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**Research Article**

# Evaluation of anti-gastric cancer of ethanol extract of *calycophyllum spruceanum* against methyl niroso ureas-NaCl induced gastric cancer in experimental rats

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## ABSTRACT

Cancer starts with a change in one single cell. This change may be initiated by external agents and genetic factors. one of the leading diseases is found to be cancer, worldwide and affecting 7.6 million deaths in 2008. Mortality rate is majorly due to Lung, stomach, liver, colon and breast cancer. Epidemiology results the second most mortality rate is due to Gastric cancer. the primary preventive measure is increasing the standards of hygiene with significant nutrition. early detection can decrease risk for cancer. The primary treatment for gastric cancer is surgery by standardized lymphadenectomy. The present research focuses on calycophyllum spruceanum for screening of gastric cancer against methyl nitroso ureas and NACL induced gastric cancer in albino rats. Cancer is induced in rats by using nitroso urea and test group is treated with herbal extracts of different doses 100, 200 and 400 mg/kg. The animals are screened for enzymes namely AST, ALT, tissue peroxidase, reduced glutathione, catalase and stomach tissue microscopic study. Results proved that the plant have gastric cancer activity.

Keywords: gastric cancer, epidemiology, classification, risk factors, treatment

## INTRODUCTION

The foremost types of cancer with utmost mortality rates are Lung, breast, colorectal, stomach or gastric cancer, at this time the 4thmost regular kind of cancer global has remained an imperative malignant disease with noteworthy ethnic, geographical, and socioeconomic differences in distribution. It is the 2ndmost familiar cause of death from cancer; just about 1 million cases of stomach cancer were documented in 2018, accounting for just about 8 percent of all the new cancer cases. It is envisaged that the number of cases will go up to 1.7 million by the year 2030.

**Causes of Gastric Cancer:** Gastric cancer is strongly connected with dietary features and Helicobacter pylori infection. Prior studies have reported that eating salty foods and N-nitro-so compounds and low ingestion of fresh fruits and vegetables increase the menace of gastric cancer. Hypertonic NaCl solutions provoke gastric cancer

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in animal models by the enhancement of tissue damage consequential in cell proliferation.

## MATERIALS AND METHODS

### Plant Collection and Extraction

i) Fresh plant material, aerial parts were collected and authenticated by registered botanist Dr. Madhava Chetty, SVU, Tirupati. Then the plant material was extracted using ethanol solvent by utilizing a soxhlet extractor until the color in the siphon tube become colorless. Collected liquid extract was filtered and extract concentrated by using Rotaevaporator. The resultant extract was packed in a container and stored in the refrigerator.
ii) After extraction phytochemical screening and bioactive constituent characterization studies were done utilizing suitable methods.

### Qualitative analysis methods

**Preliminary Phytochemical Screening:** The collected extracts were subjected to phytochemical screening using freshly prepared reagents to analyze the present phytoconstituents in extracts. The extracts were analyzed for the detection of alkaloids, glycosides, flavonoids, proteins and amino acids, tannins, terpenoids, phenolic, saponins, fixed oils carbohydrates and gum and mucilage.

**Experimental animals**: Male Wistar rats with one and half month age were used for the study. All experimentation and procedures carried out on the animals (rats) were approved by the Institutional Ethics Committee. Rats were placed in a room at 25 ± 1◦C under standard mentioned conditions (12-h dark-light cycle). They were isolated in a polypropylene cage and offered food and water ad libitum. Animals were quarantined and become accustomed to laboratory conditions for 7 days proceeding to study initiation. Animals were observed for general health and appropriateness for testing during this phase. (Approval number: 006/IAEC/NCPA/M.PHARMACY19-20 )

**Experimental Induction of gastric Cancer**: To induce gastric cancer, all groups animals except vehicle control are treated with 100mg/kg MNU (methyl nitrosourea) on 0 and 14th (dissolved in citrate buffer, pH 4.5) day using intra-gastric tube and first 3 days of every week treated with s-NaCl (30% NaCl solution, 1 mL/rat) (oral route) for 4 weeks. For the Invivo model, the animals were divided into six groups. Each group contains six animals. For ethanol extract grouping is as follows:

**Table 1: Experimental Induction of gastric Cancer**

| |
|---|
| Group 1: Normal Group (Tween 80) |
| Group 2: Disease Control Group- methyl nitrosourea 100mg/kg on 0 and 14 days of experiment+s-Nacl (1mL/rat twice a week for 3 weeks, P.O.) |
| Group 3: standard-MNU+Docetaxel injection on 0 and 14 days of experiments |
| Group 4: Test-I-*Calycophyllum spruceanum* 100 mg/kg + methyl nitrosourea 100mg/kg on 0 and 14 days of experiment+s-Nacl (1mL/rat twice a week for 3 weeks, P.O.) |
| Group 5: Test II-*Calycophyllum spruceanum*200 mg/kg + methyl nitrosourea 100mg/kg on 0 and 14 days of experiment+s-Nacl (1mL/rat twice a week for 3 weeks, P.O.) |
| Group 6: Test III-*Calycophyllum spruceanum*400 mg/kg + methyl nitrosourea 100mg/kg on 0 and 14 days of experiment+s-Nacl (1mL/rat twice a week for 3 weeks, P.O.) |

The study duration is 20 weeks

### Physical and Biochemical parameters analysis

Bodyweight and food intake

### Tissue preparation and homogenization:

Animals were sacrificed through decapitation with help of light ether anesthesia. Stomach tissue & liver were removed, washed thoroughly with ice-cold normal saline and weighed properly. Then without delay tissues were dipped in liquid nitrogen for 30 sec to stop any enzymatic metabolic action and stored at -80°c for additional processing. A fraction of liver and stomach was taken, pulverized into small pieces and homogenized with a homogenizer in ice-cold phosphate buffer saline (PBS) (0.1M, $p^H$ 7.4) to obtain 1:9 (w/v) (10%) full homogenate. A fraction of the homogenate was taken and mixed with an equivalent volume of 10% Trichloroacetic acid (TCA) and centrifuged at 5000 rpm for 10 min and the supernatant was utilized for the measurement of GSH, MDA. The left-behind fraction was centrifuged at 17,000g for 60 min at 4 °C, and supernatants were utilized for the assay for estimation of protein, CAT, GSH, SOD, and ROS**.**

### Histology

- Stomach
- Liver.

**Histological examination of Liver and Tumor**: From each rat, the segment of the liver and parts of tumor tissue were fixed in 10% formalin solution and embedded in paraffin after custom tissue processing. Longitudinal sections (5 μm) were prepared and stained with Hematoxylin and eosin to inspect the alteration in cellular morphology.

**Estimation of antioxidant enzyme levels:** Tissue (liver, plasma and stomach) homogenate was used to estimate thiobarbituric acid reactive substances (TBARS) and level of antioxidant enzymes, *viz.* CAT, GSH, SOD, and ROS.

**Estimation of lipid peroxidation:** Quantitative measurement of TBARS, an index of lipid peroxidation in liver, plasma and stomach was performed according to the method of Supernatant of homogenate (0.2 ml) was pipette out in a test tube, followed by addition of 8.1% sodium dodecyl sulfate (0.2 ml), 30% acetic acid (pH 3.5; 1.5 ml), and 0.8% of thiobarbituric acid (1.5 ml). The volume of the reaction mixture was attained with distilled water, up to 4 ml and warmed for 1 hour at 95°C. Cooled the solution to room temperature and distilled water (1 ml) following the addition of n-Butanol-pyridine mixture (5 ml; 15:1 *v/v*). After mixing, the solution was centrifuged at 4000 rpm for 10 minutes. The organic layer was recovered to estimate the absorbance at 532 nm. The comparison was carried out between the absorbance of extract and the MDA standard. The final concentration was expressed as nanomoles per mg of protein.

**Biochemical assays:** Biochemical tests for liver functions through assessment of liver enzymes such as Alanine aminotransferase Auto blood analyzer (ALT Dimension x and, Siemens, USA), Aspartate aminotransferase(AST Dimension x and, Siemens, USA,) were carried out.

**A. Principle of ALT:** Alanine aminotransferase (ALT) catalyzes the transamination of L-alanine to α-ketoglutarate (α-KG), forming L-glutamate and pyruvate the pyruvate produced is reduced to lactate through lactate dehydrogenase (LDH) with concurrent oxidation of reduced nicotinamide – adenine dinucleotide (NADH). The alteration in absorbance is directly proportional to the activity of ALT and is measured using a bichromatic (340.700 nm) rate procedure.

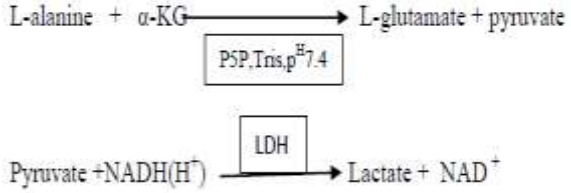



**Figure 1: Principle of ALT**

**B. Principle of AST:** Aspartate aminotransferase (AST) catalyzes the transamination of L-aspartate to α-ketoglutarate, making L-glutamate and oxaloacetate. The Oxaloacetate produced is reduced to malate through malate dehydrogenase (MDH) with concurrent oxidation of reduced nicotinamide –adenine dinucleotide (NADH). The alteration in absorbance with time owing to the alteration of NADH to NAD is directly proportional to the activity of AST and is measured through a bichromatic (340.700 nm) rate procedure.

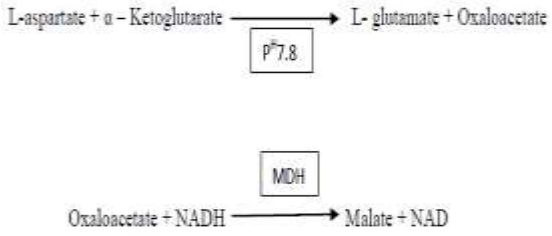



**Figure 2: Principle of AST**

**Measurement of oxidative stress markers:**

**C. Estimation of Total reactive oxygen species:** ROS in tissues of the stomach and liver were determined fluorometrically through 2,7-dichlorofluorescein diacetate (DCF-DA) as the technique specified.

**Principle**:

2,7-dichlorofluorescein diacetate (DCF-DA), a non-fluorescent ester easily taken up employing cells through passive diffusion crosswise plasma membrane. On entering cells, DCF-DA is de-esterified through enzyme esterase to the ionized oxidant sensitive free acid(DCFH), reacts with ROS to produce the fluorescent 2,7-dichlorofluorescein(DCF) and fluorescence was measured at 488nm, an excitation wavelength and 525 nm, emission wavelength.

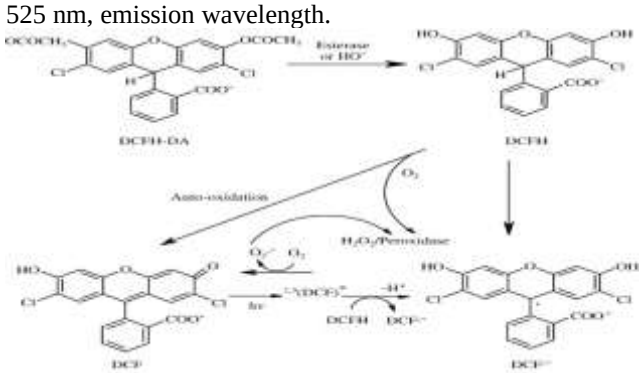



**Figure 3: Principle of DCF-DA**

**Reagents**:

| |
|---|
| a.100 uM DCF-DA(dissolved in DMSO) |
| b. phosphate buffer saline(PBS) (0.1M,Ph 7.4) |
| c.Tissuehomogenate(10% w/v) |

**Methodology**:
10ul of 100 Um DCFHDA (DMSO dissolved) was added to 90 ul of supernatant and incubated for about 30 min at RT in the dark. Post incubation, the volume was made up to 3ml utilizing PBS (0.1M, pH 7.4) and fluorescence was measured at 525nm an excitation wavelength utilizing a microplate reader. The result was represented as a % change of fluorescence where the normal group was taken as 100%

**D. Estimation of Tissue lipid peroxides**
The method used for measuring plasma lipid peroxidation using the method of Oh Kwa et al 1979.

**Principle**
The lipids present in the cell of small units form Malonaldehyde. The produced malonaldehyde reacts with Thiobarbituric acid (TBA) to create Thiobarbituric Acid Reacting Substance (TBARS), a pink color substance with 532 nm absorption maxima with 156mM$^{-1}$ cm$^{-1}$ extinction coefficient.

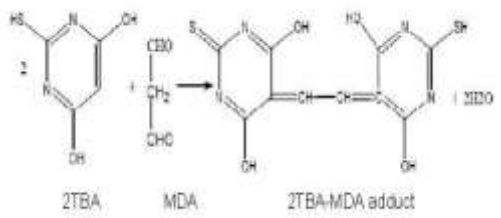



**Figure 4: Mechanism of 2TBA- MDA Product**

**Procedure**
To 100 µl of tissue or plasma sample 100 µl of ice-cold 10%TCA and centrifuged at 10000 rpm and to 100 µl of obtained supernatant 200 µl of 8.1% SDS, 1.5 ml of 20% Glacial acetic acid and 1.5 of 8.1% TBA was added in the similar order. The tubes were enclosed with glass marbles and placed in boiling water for 1 hr at 95$^0$C. Post cooling the absorbance was measured at 532 nm.
The MDA content is represented as tissues nmol/mg protein and for nmol/ml of plasma

**Reagents**

| | |
|---|---|
| 1) Glacial Acetic acid | 20% |
| 2) Thiobarbituric acid | 0.8% |
| 3) SDS | 8.1% |

**E. Estimation of Reduced Glutathione**
Tri peptide Glutathione is most plentiful on enzymatic anti-oxidant. Universally present in all cells it turns as a substrate for antioxidant enzymes like Glutathione Peroxidase and Glutathione-s-Transferase have a role in detoxification of Organic hydroperoxides, hydrogen peroxides and Xenobiotics.
The total reduced glutathione was measured employing Ellmann modified method.

**Principle**
Glutathione reacts with Dithio bis nitro Benzoic acid (DTNB) compound to produce compounds that absorb greatly at 412 nm wavelength.

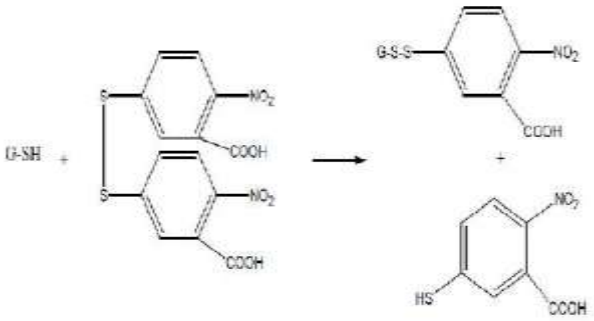



**Figure 5: Principle of GSH Assay**
**Reagents**

1) Phosphate Buffer 0.2M pH 7.6 50 ml of reagent (a) and 42.4ml of reagent (b) were added and up to 200ml with distilled water is added
2) DTNB 19.8mg in 50ml of reagent 1
3) Glutathione Standard 15.375mg of GSH solubilized in 50 ml of 0.1N HCl solution

**Procedure**
To 2.3 ml of Phosphate buffer, 0.2 ml of plasma or tissue was added followed by 0.5 ml of DTNB reagent the yellow color established was measured at 412 nm wavelength. The GSH amount is represented as mol of GSH /ml of plasma. And for tissues is represented as mol/mg protein.

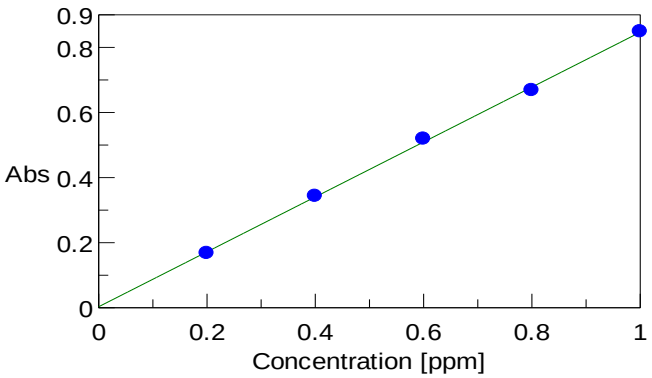



**Figure 6: Standard graph of GSH**

**F. Estimation of Catalase (CAT):**
Catalase activity in plasma and tissue samples was estimated using the decomposition rate of hydrogen peroxide at 240 nm, according to the technique specified by Aebi. et al., 1974.
**Principle**
Catalase is a very common enzyme in all living organisms that catalyze the breakdown of hydrogen peroxide to water and oxygen and activity was estimated by measuring the decomposition rate of hydrogen peroxide at 240 nm wavelength. The

change in absorbance (ΔE240) per unit time is a measure of the activity of catalase.

$$2H_2O_2 \text{ --Catalase--> } 2H_2O + O_2$$

**Reagents:**

1. Phosphate Buffer (50mM, pH 7) Mix both solutions in 1:1.55 (a: b).

| |
|---|
| (a): Dissolve 6.81 g $KH_2PO_4$ in double distilled water to make 1000ml. |
| (b): Dissolve 7.091 g $Na_2HPO_4$ in double distilled water to make 1000ml. |

2. Hydrogen Peroxide (30mM) (Dilute 0.34 ml of 30% $H_2O_2$ with phosphate buffer till 100 ml)

**Procedure:**

To the 1.95ml phosphate buffer (50mM, pH 7), 50µL of experimental Sample were mixed. Then alteration in absorbance were measured at 240nm by means of addition of 1ml hydrogen peroxide (30mM) for 60 sec at 15sec interval and then the activity was estimated by following formula,

Catalase activity (k/min) = $(1/\Delta t) \times \ln (s1/s2) = (2.3/\Delta t) \times \log (s1/s2)$

Where, $\Delta t = t2 - t1$ (Time interval)

s1 and s2 = H2O2 concentrations at times t1 and t2.

**G. Estimation of Superoxide dismutase (SOD):**

Total SOD activity (cytosolic and mitochondrial) was estimated utilizing the SOD assay kit (Sigma-Aldrich Co, St Louis, MO, USA) based on manufacturer specifications.

**Principle**

The method relies on the enzymatic production of superoxide radicals through Xanthine and Xanthine oxidase (XO) reacts with water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)- 3-(4-nitrophenyl)-5-(2,4- disulfophenyl)- 2H-tetrazolium, monosodium salt) make a water-soluble formazan dye upon reduction reaction with a superoxide anion. Since the absorbance at 450 nm is proportionate to the quantity of superoxide anion, the SOD activity as an inhibition activity may be quantified by determining the reduction in the development of color at 450 nm.

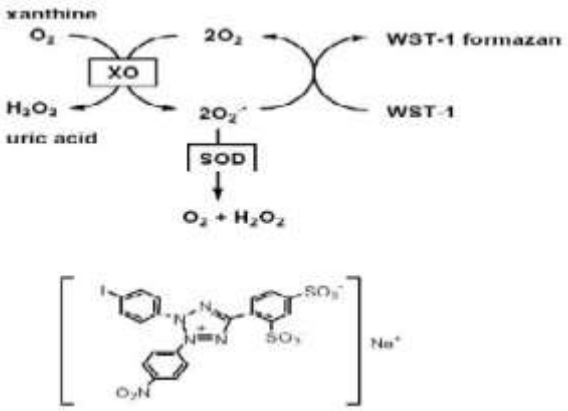



**Figure 7: Mechanism of SOD**

**Reagents:**

Reagents of SOD assay kit (Cat. no-19160-1KT-F).

**Procedure**:

96-Well plate was utilized for the SOD activity estimation. Based on manufacturer instruction, three blanks as blank 1, blank 2 and blank 3 were engaged in duplicate wells. To each blank 1 and blank 3 well, 20µl of double distilled water and 20µl of sample solution to each sample and blank 2 well were mixed. WST Working Solution (200µl) was added to each well and mixed and then 20µl of Dilution Buffer to each of blank 2 and blank 3 well. At last 20µl of Enzyme, Working Solution was added to each one of sample and blank 1 well, mixed methodically. Incubation of the plate is done at 37 °C for 20 min. Read the absorbance at 450 nm employing a microplate reader. SOD activity (inhibition rate %) was estimated through the following equation:

SOD activity (inhibition rate %) = {[(Ablank1 - Ablank3)–(A sample - Ablank2)]/ (Ablank1-Ablank3)} x 100

**RESULTS**

**The percentage yield of ethanolic extract of Calycophyllum spruceanum**

**Table 2: Percentage yields of Extracts**

| S no | Solvent | Percentage Yield |
|---|---|---|
| 1. | Ethanol | 11.45% |

**Phytochemical component observed in ethanolic extract of Calycophyllum spruceanum**

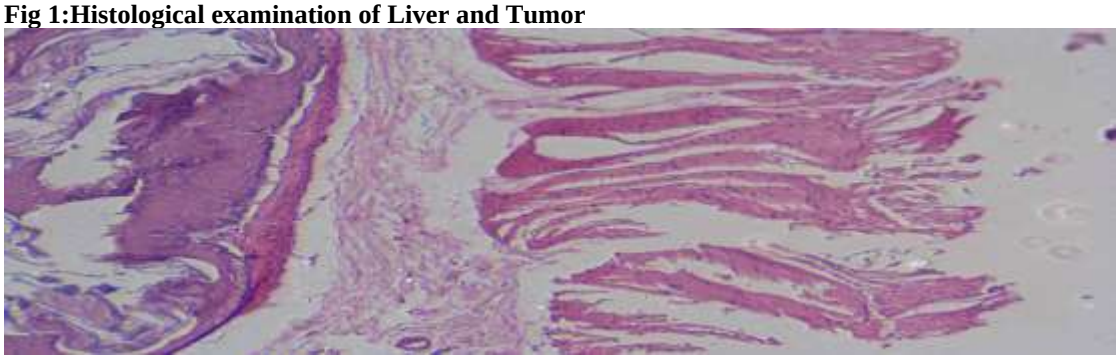
**Table 3: Particulars of Qualitative Phytochemical Assessment**

| S. No. | Test | Ethanolic Extract |
|---|---|---|
| **1** | **Alkaloids** | |
| | Mayer's test | + |
| | Dragondraffs Test | + |
| | Hager's Test | + |

| | | |
|---|---|---|
| | Wagner's test | + |
| **2** | **Carbohydrates** | |
| | Mohlish's test | - |
| **3** | **Reducing Sugars** | |
| | Fehling's test | + |
| | Benedicts Test | + |
| **4** | **Saponins** | |
| | Foam test | - |
| | Fourth Test | - |
| **5** | **Phytosterols** | |
| | Salkowski Test | + |
| | LibermanBurchard's Test | + |
| **6** | **Phenolics** | |
| | Ferric chloride test | - |
| | Lead acetate test | - |
| **7** | **Tannins** | |
| | Ferric chloride test | + |
| **8** | **Flavones and Flavonoids** | |
| | Lead Acetate Test | + |
| | Alkaline Reagent Test | + |
| **9** | **Glycosides** | |
| | Keller killliani test | + |
| **10** | **Proteins and amino Acids** | |
| | Ninhydrin test | - |
| | Biuret test | - |
| **11** | **Terpenoids** | |
| | Salkowskis Test | + |
| **12** | **Fixed oils and fats** | |
| | Spot test | - |
| | Saponification Test | - |
| **13** | **Gum and Mucilages** | |
| | Ruthenium Red Solution | + |

**Histological examination of Liver and Tumor**

**Fig 1:Histological examination of Liver and Tumor**



Microscopic (H&E staining, 100x) appearance of squamous cell carcinoma (SCC) in the forestomachs. The multiple polyploid tumors developed in the stomach after MNU and saturated NaCladministration. SCCs in the gastric epithelium shows the invasion of cancerous tissue with dyskeratosis through the submucosal layer

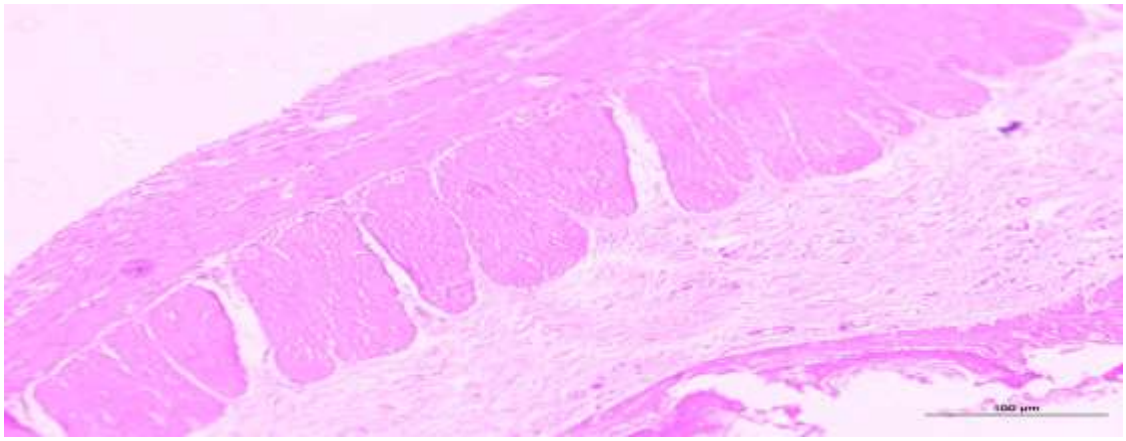


**Figure 2: CONTROL**

Microscopic (H&E staining, 100x) appearance of the epithelial squamous layer in the stomachs of a control group

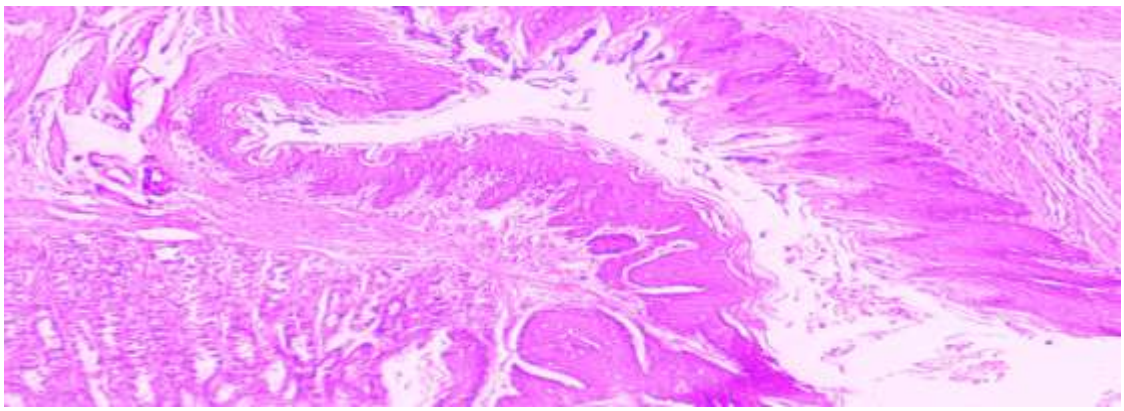


**Figure 3: MNU+CS 100 mg/kg**

Microscopic (H&E staining, 100x) appearance of squamous cell carcinoma (SCC) in the forestomachs. The multiple polypoid tumors developed in the stomach after MNU and saturated NaCl administration, SCCs in the gastric epithelium show the invasion of cancerous tissue with dyskeratosis through submucosal layer decreased when compared with MNU group which are treated with docetaxel and CS.

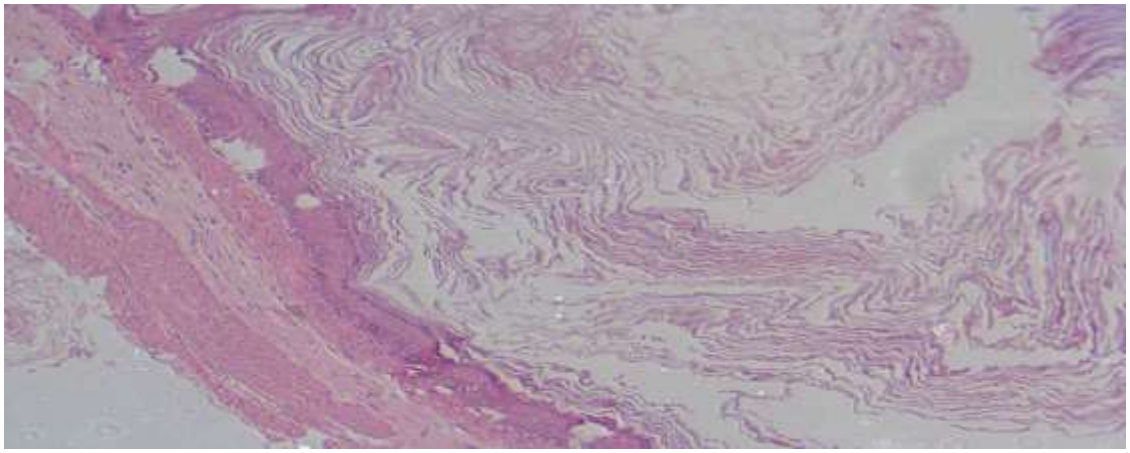


**Figure 4: MNU+CS 200 mg/kg**

Microscopic (H&E staining, 100x) appearance of squamous cell carcinoma (SCC) in the forestomachs. The multiple polypoid tumors developed in the stomach after MNU and saturated NaCl administration, SCCs in the gastric epithelium show the invasion of cancerous tissue with dyskeratosis through submucosal layer decreased when compared with MNU group which are treated with docetaxel.

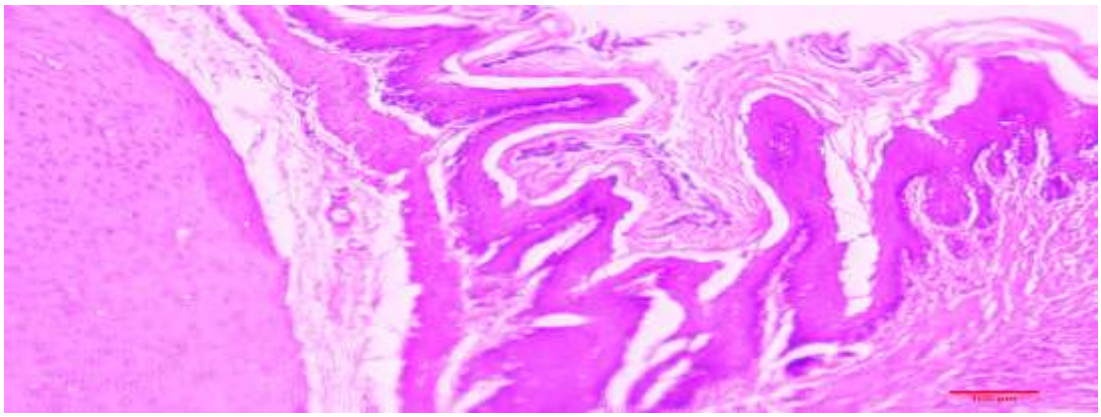


**Figure5:MNU+CS 400 mg/kg**

Microscopic (H&E staining, 100x) appearance of squamous cell carcinoma (SCC) in the forestomachs. The multiple polypoid tumors developed in the stomach after MNU and saturated NaCl administration, SCCs in the gastric epithelium show the invasion of cancerous tissue with dyskeratosis through submucosal layer decreased when compared with MNU group which are treated with docetaxel.

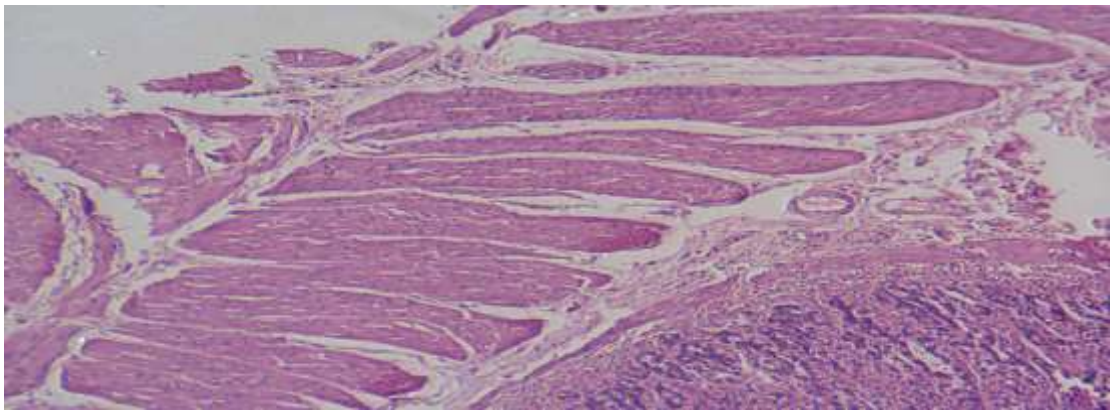


**Figure 6:MNU+DOCETAXEL**

Microscopic (H&E staining, 100x) appearance of the epithelial squamous layer in the fore stomachs of CS group.

**LIVER HISTOPATHOLOGY:**

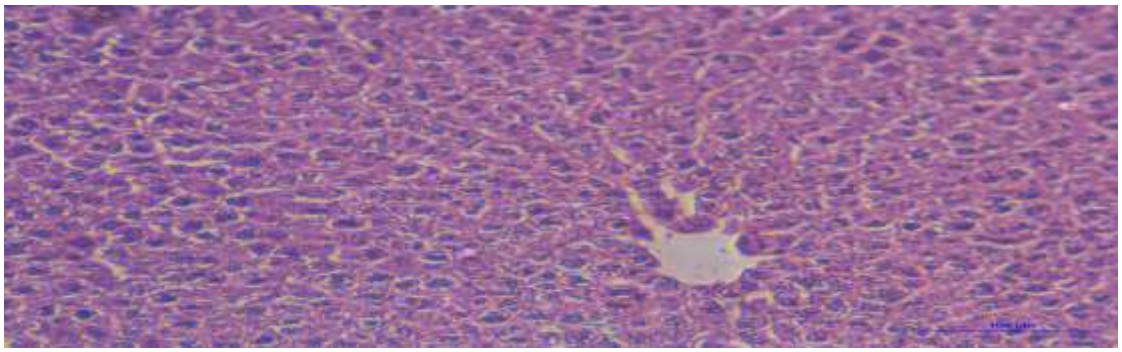


**Figure 7:CONTROL**

Histologic appearance of rat liver from the control group. The normal lobular structure is seen. Central vein and hepatocytes (Hematoxylin-eosin, 100x).

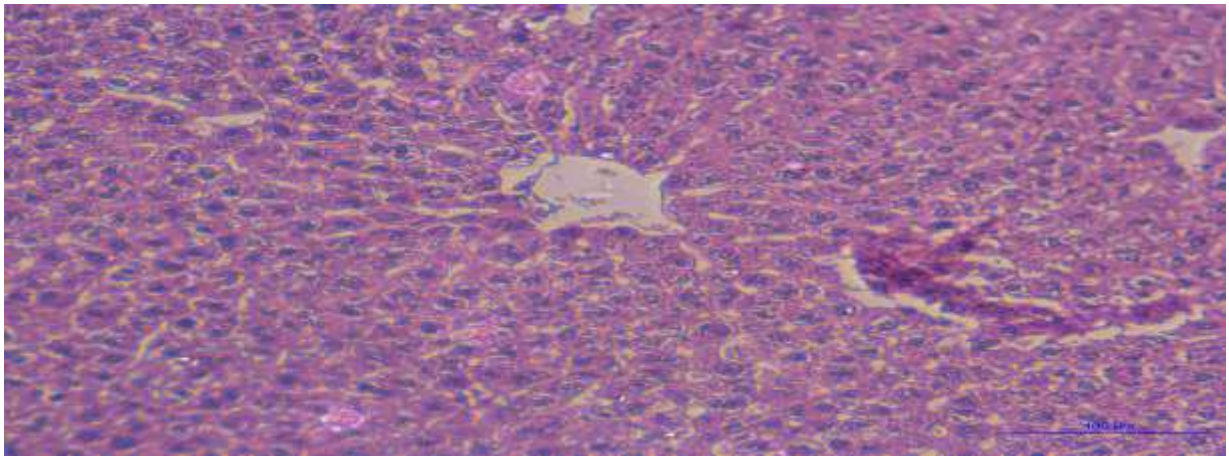


**Figure 8:MNU**

Histologic appearance of rat liver from the MNU group. The normal lobular structure is seen. Central vein and hepatocytes with very less necrosis (Hematoxylin-eosin, x 100).

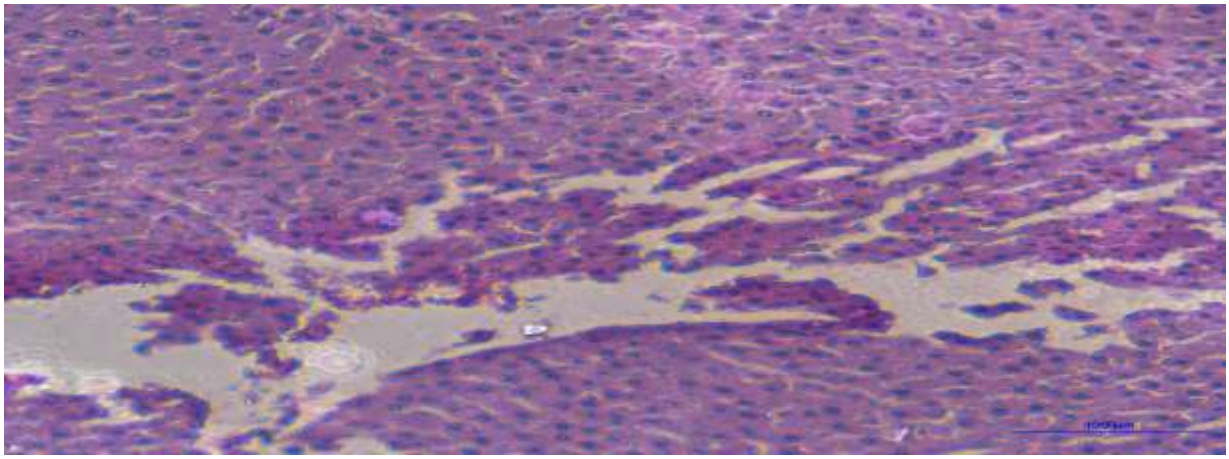


**Figure 9:MNU+DOCETAXEL**

High dose MNU and Docetaxel administered group. Bile duct proliferation in portal area, enlargement in a periportal area with mononuclear cell infiltration and parenchymal cell degeneration are seen (Hematoxylin-eosin, x 100)

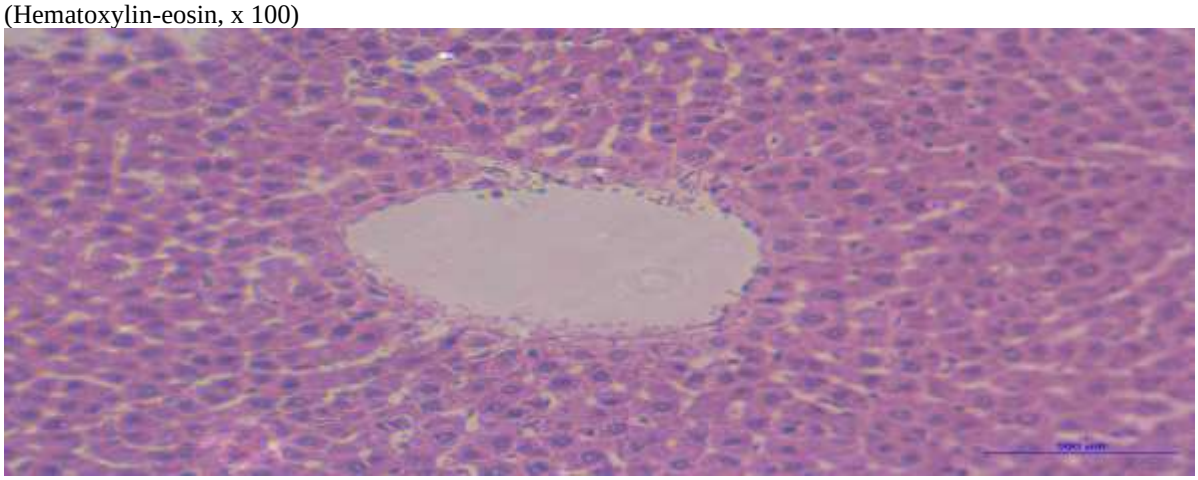


**Figure 10:MNU+CS 100 mg/kg**

Higher magnification of liver lobule in MNU and Docetaxel and CS. Parenchymal cell necrosis, sinusoidal dilatations, hepatocytes with a pyknotic nucleus and eosinophilic cytoplasm High dose MNU and Docetaxel and CS administered group (Hematoxylin-eosin, x 100)

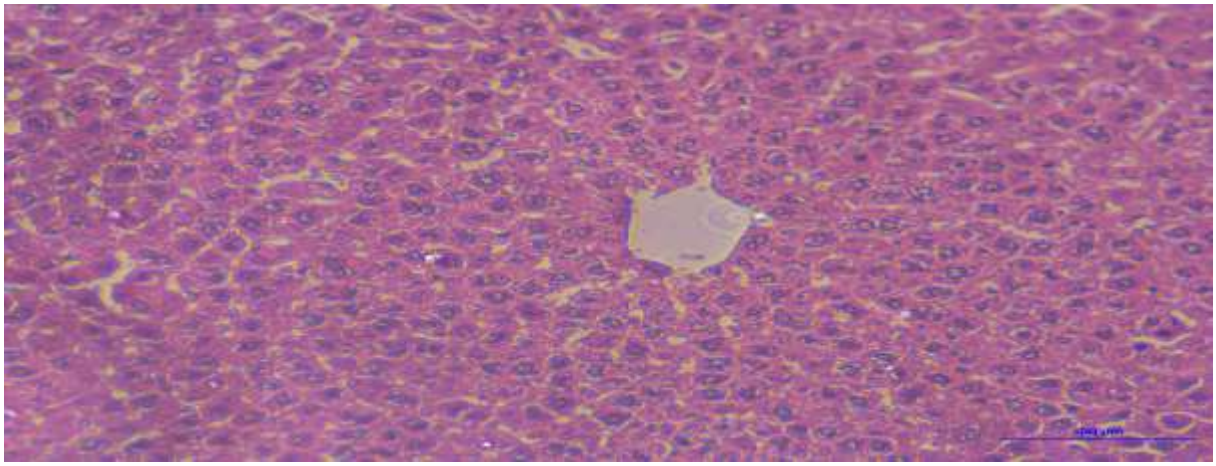


**Figure 11:MNU+CS 200 mg/kg**

Histologic appearance of rat liver from the control group. The normal lobular structure is seen. Central vein and hepatocytes (Hematoxylin-eosin, 100x).

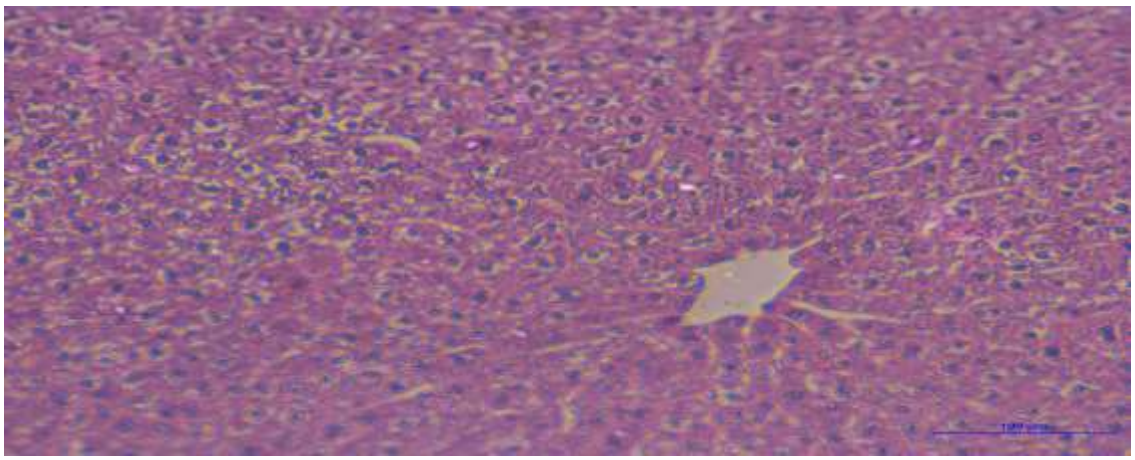


**Figure 12: MNU+CS 400 mg/kg**

Histologic appearance of rat liver from the control group. The normal lobular structure is seen. Central vein and hepatocytes (Hematoxylin-eosin, 100x).

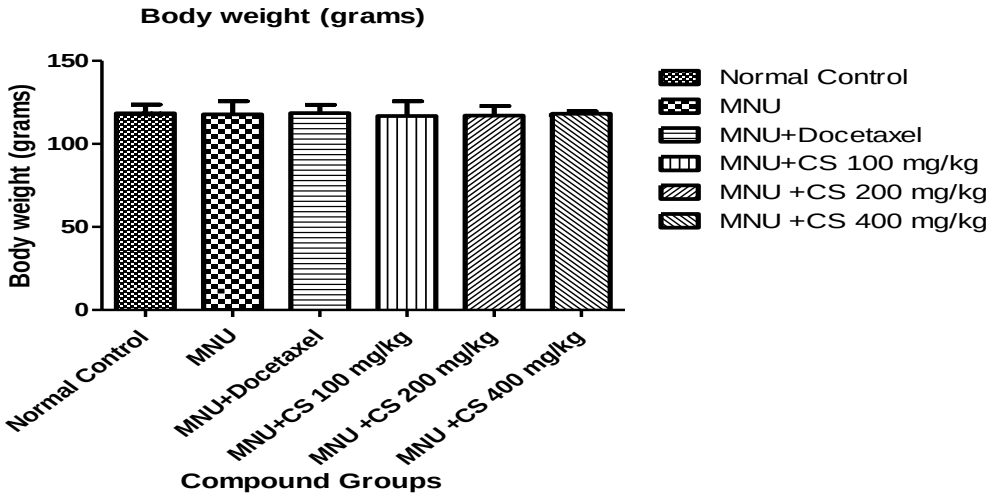



**Figure 13: The Bodyweight on 0 days of the MNU Induced Study**

The figure shows the Bodyweight on 0 days of the MNU induced study. Values are mentioned as Mean ± S.E.M. No statistical difference was observed in the parameter.

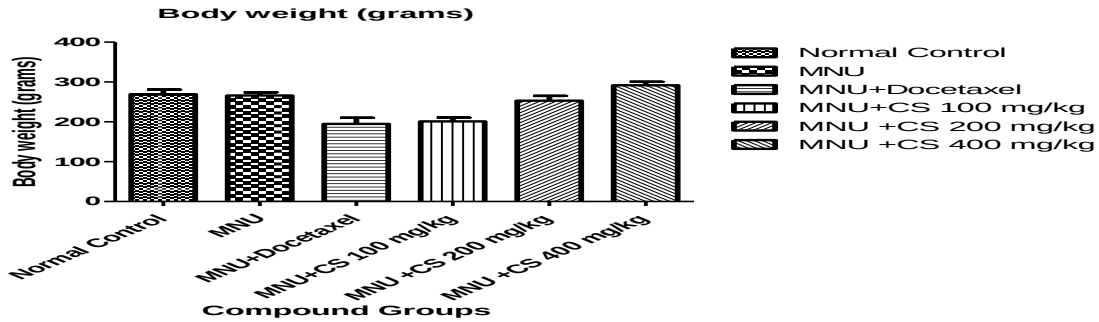



**Figure 14: After Treatment Body Weight**

**Table 7: Table Showing Weight Variation In Animals Before And After Treatment:**

| Compound Groups | Before Treatment Bodyweight (grams) | After Treatment Bodyweight (grams) |
|---|---|---|
| Normal Control | 118.45±12.82 | 269.53 ±23.5 |
| MNU | 117.86±19.2 | 266± 19.4 |
| MNU+ Docetaxel | 118.61±12.08 | 194.72± 22.1** |
| MNU+ CS 100 mg/kg | 116.8±21.68 | 201.2 ± 18.6* |
| MNU + CS 200 mg/kg Animal Body weights before and after treatment | 117.05±14.1 | 253.08±22.1 |
| MNU +CS 400 mg/kg | 118.2±3.6 | 291.81±20.6*** |

Values are mean ± SD of three replicates (n=6). Where a represents #P < 0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+ docetaxel.

**Effect of CS and its combinations on Biochemical parameters:**

**ALT VALUES ON 0th DAY:**

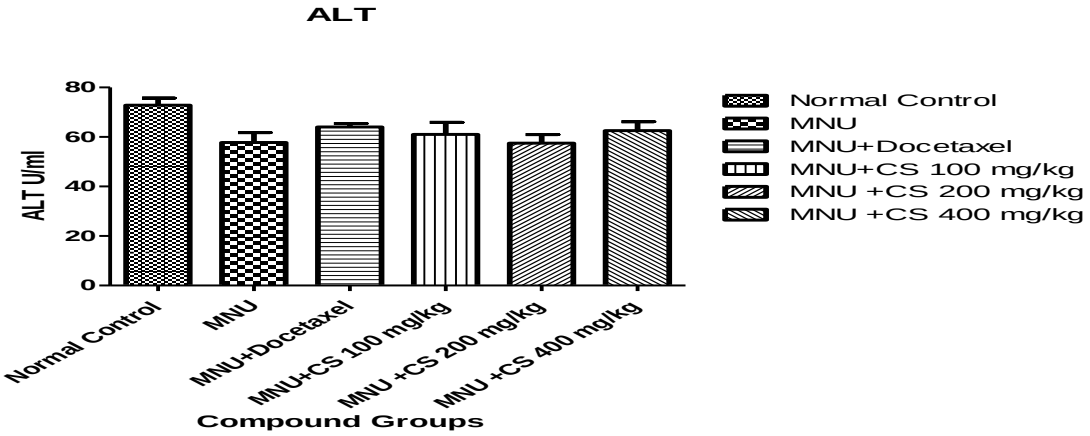



**Figure 15: ALT Values On 0th Day**

The figure shows the different biochemical parameters on 0 days of the MNU induced study. ALT Values are mentioned as Mean ± S.E.M. No statistical difference was observed in the parameter.

**AST VALUES ON 0th DAY**

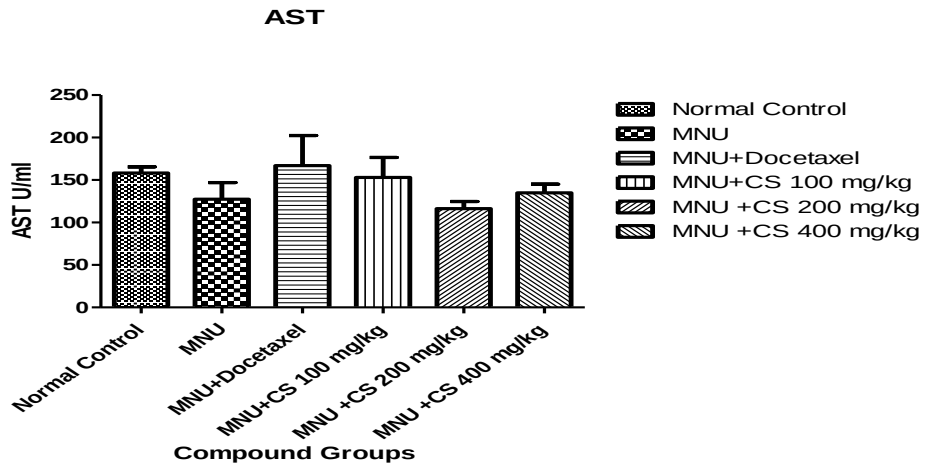



**Figure 16 :AST Values On 0thday**

The figure shows the different biochemical parameters on 0 day of the MNU induced study. AST Values are mentioned as Mean ± S.E.M. No statistical difference was observed in the parameter.

**AST VALUES AFTER TREATMENT:**

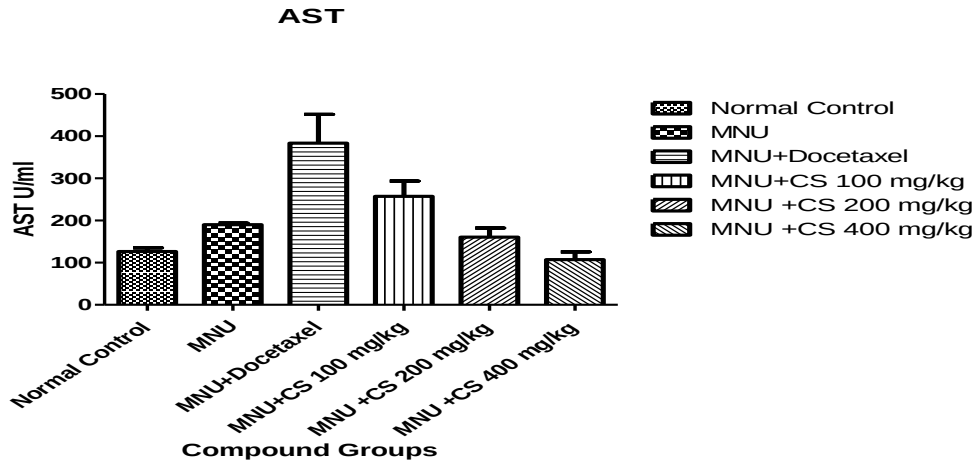



**Figure 17: AST Values After Treatment**

**ALT VALUES AFTER TREATMENT:**

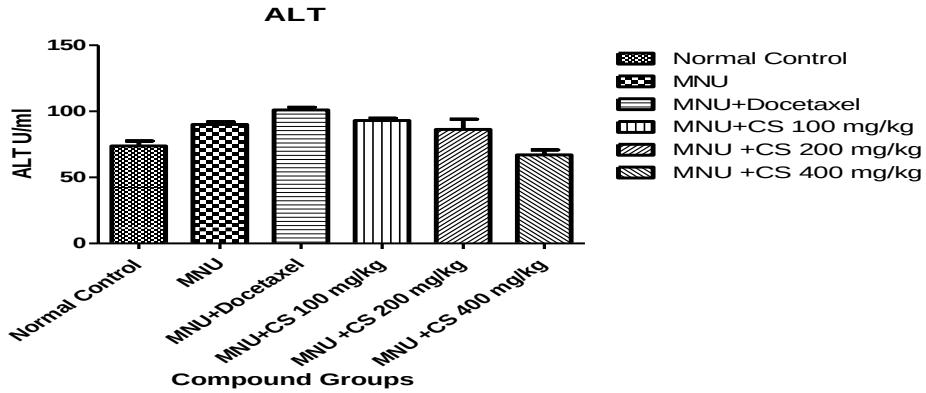



**Figure 18: ALT Values After Treatment**

**Table 8: ALT Values Before And After Treatment**

| **Compound Groups** | **Before Treatment ALT U/ml** | **After Treatment ALT U/ml** |
|---|---|---|
| Normal Control | 72.81±6.41 | 73.83 ±8.99 |
| MNU | 57.8±8.9 | 90± 2.82 |
| MNU+Docetaxel | 64±3.24 | 101± 2.82*** |
| MNU+CS 100 mg/kg | 61±9.83 | 93± 3.7 |
| MNU +CS 200 mg/kg | 57.5±6.95 | 86±15.73* |
| MNU +CS 400 mg/kg | 62.6±7.92 | 67±7.74** |

Values are mean ± SD of three replicates (n=6). Where a represents #P < 0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Table 9: AST Values Before And After Treatment**

| Compound Groups | Before Treatment AST U/ml | After Treatment AST U/ml |
|---|---|---|
| Normal Control | 158.2±16.1 | 126.66±20.79 |
| MNU | 127.4±23.6 | 190±25.65 |
| MNU+Docetaxel | 167±30.91 | 383.66± 38.15*** |
| MNU+CS 100 mg/kg | 153±27.13 | 257.2± 41.16 |
| MNU +CS 200 mg/kg | 116.25±17.05 | 160.5±23.85* |
| MNU +CS 400 mg/kg | 134.8±22.97 | 107.2±21.58** |

Values are mean ± SD of three replicates (n=6). Where a represents #P < 0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on lipid peroxidation:**
**Liver:**

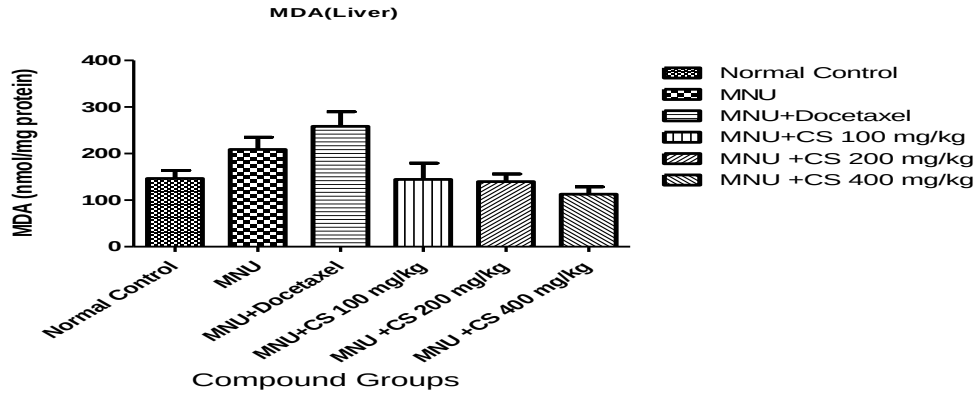



**Figure 19 :Effect of CS On Lipid Peroxidation-Liver**

**Table 10: TBARS After Treatment – Liver**

| **Compound Groups** | **TBARS Assay(nmol/mg protein)** |
|---|---|
| Normal Control | 146.36±24.84 |
| MNU | 208.74± 27.57 |
| MNU+Docetaxel | 258.49±22.69*** |
| MNU+CS 100 mg/kg | 144.53± 28.02 |
| MNU +CS 200 mg/kg | 139.92±20.12* |
| MNU +CS 400 mg/kg | 112.8±28.2** |

Values are mean ± SD of three replicates (n=6). Where a represents #P < 0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Plasma:**

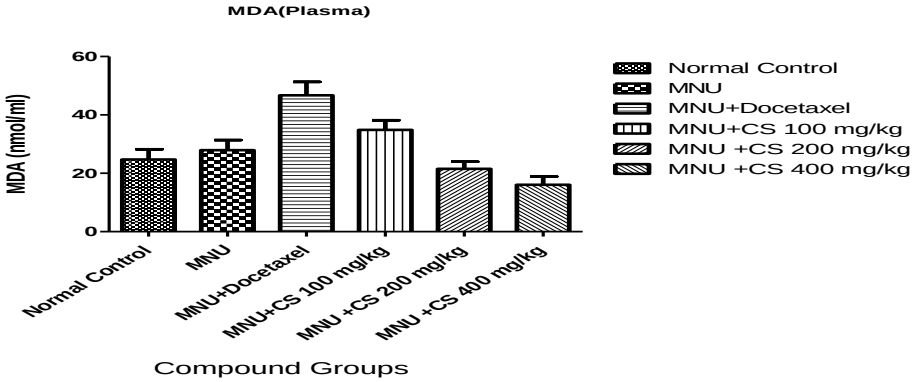



**Figure 20 : Effect of CS on Lipid Peroxidation-Plasma**

**Table 11: TBARS Assay After Treatment -Plasma**

| Compound Groups | TBARS Assay(nmol/ml) |
|---|---|
| Normal Control | 24.7± 7.05 |
| MNU | 27.91± 6.92 |
| MNU+Docetaxel | 46.74±9.11*** |
| MNU+CS 100 mg/kg | 34.89±6.55 |
| MNU +CS 200 mg/kg | 21.53±4.89* |
| MNU +CS 400 mg/kg | 16.06±5.6** |

Values are mean ± SD of three replicates (n=6). Where a represents #P < 0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Stomach:**

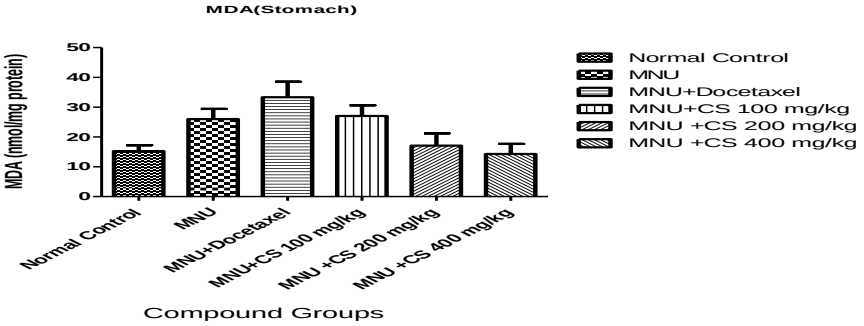



**Figure 21: Effect of CS on Lipid Peroxidation-Stomach**

**Table 12: TBARS Assay After Treatment -Stomach**

| Compound Groups | TBARS Assay(nmol/mg protein) |
|---|---|
| Normal Control | 15.25±4.84 |
| MNU | 26.03± 7.57 |
| MNU+Docetaxel | 33.36±12.69*** |
| MNU+CS 100 mg/kg | 27.05± 8.02 |
| MNU +CS 200 mg/kg | 17.11±10.12* |
| MNU +CS 400 mg/kg | 14.3±8.2** |

Values are mean ± SD of three replicates (n=6). Where a represents #P < 0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on Glutathione levels:**
**Effect of CS on Glutathione levels in liver tissue:**

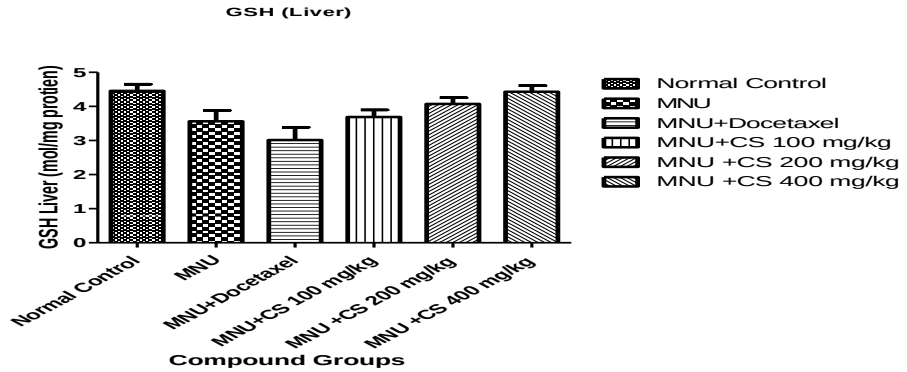



**Figure 22: Effect of CS on Glutathione Levels In Liver Tissue**

**Table 13: GSH Assay-Liver**

| Compound Groups | GSH(mol/mg protein) |
|---|---|
| Normal Control | 4.45±0.47 |
| MNU | 3.56± 0.7 |
| MNU+Docetaxel | 3.01±0.91* |
| MNU+CS 100 mg/kg | 3.68± 0.47 |
| MNU +CS 200 mg/kg | 4.07±0.44** |
| MNU +CS 400 mg/kg | 4.43±0.44*** |

Values are mean ± SD of three replicates (n=6). Where a represents #P < 0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on Glutathione levels in Plasma:**

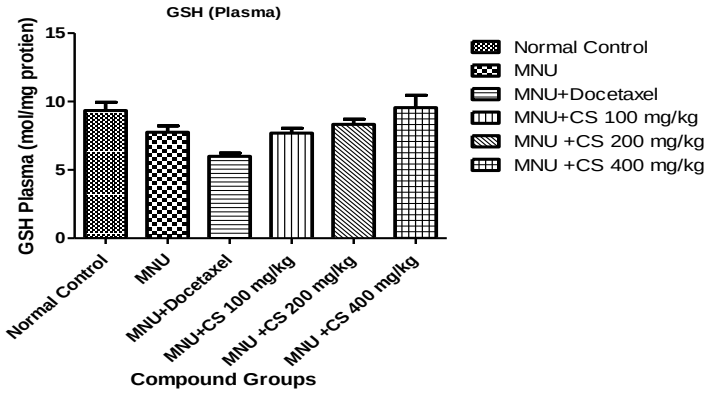



**Figure 23: Effect of CS on Glutathione levels in Plasma**

**Table 14:GSH Assay-Plasma**

| Compound Groups | GSH(mol/ml) |
|---|---|
| Normal Control | 9.3±1.19 |
| MNU | 7.75± 0.93 |
| MNU+Docetaxel | 5.99±0.47*** |
| MNU+CS 100 mg/kg | 7.69± 0.67 |
| MNU +CS 200 mg/kg | 8.33±0.73* |
| MNU +CS 400 mg/kg | 8.55±01.80** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <

0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on Glutathione levels in Stomach:**

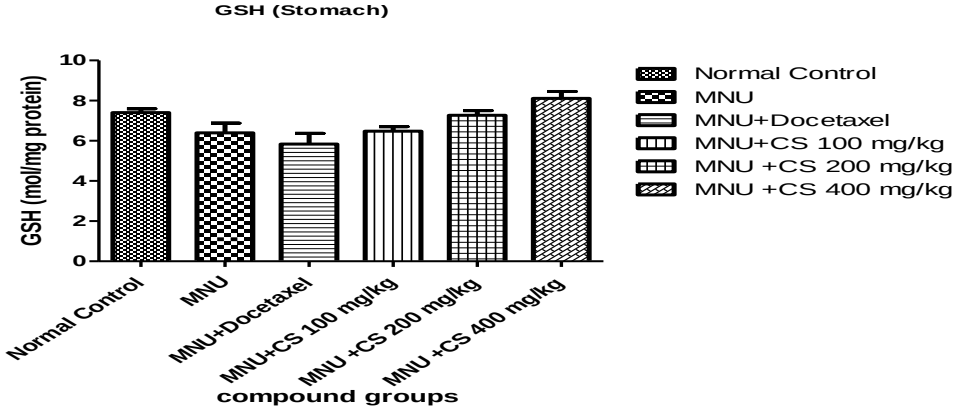



**Figure 24: Effect of CS on Glutathione levels in Stomach**

**Table 15: GSH Assay-Stomach**

| Compound Groups | GSH(mol/mg protein) |
|---|---|
| Normal Control | 7.4±0.45 |
| MNU | 6.39± 1.18 |
| MNU+Docetaxel | 5.84±1.18* |
| MNU+CS 100 mg/kg | 6.47± 0.5 |
| MNU +CS 200 mg/kg | 7.27±0.55** |
| MNU +CS 400 mg/kg | 8.11±0.82*** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on Catalase levels:**

**Effect of CS on Catalase levels in Liver:**

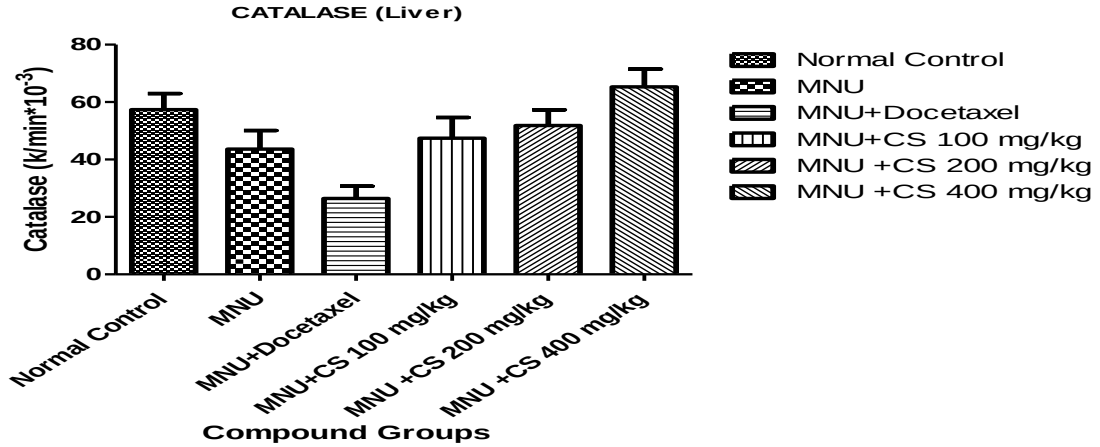



**Figure 25: Effect of CS on Catalase levels in Liver**

**Table 16:Catalase Assay-Liver**

| Compound Groups | Catalase(k/min*$10^{-3}$) |
|---|---|
| Normal Control | 57.36±13.64 |
| MNU | 43.65± 14.35 |
| MNU+Docetaxel | 26.45±10.35*** |
| MNU+CS 100 mg/kg | 47.41± 16.11 |
| MNU +CS 200 mg/kg | 51.82±13.36* |
| MNU +CS 400 mg/kg | 65.31±15.15** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <

0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on Catalase levels in Plasma:**

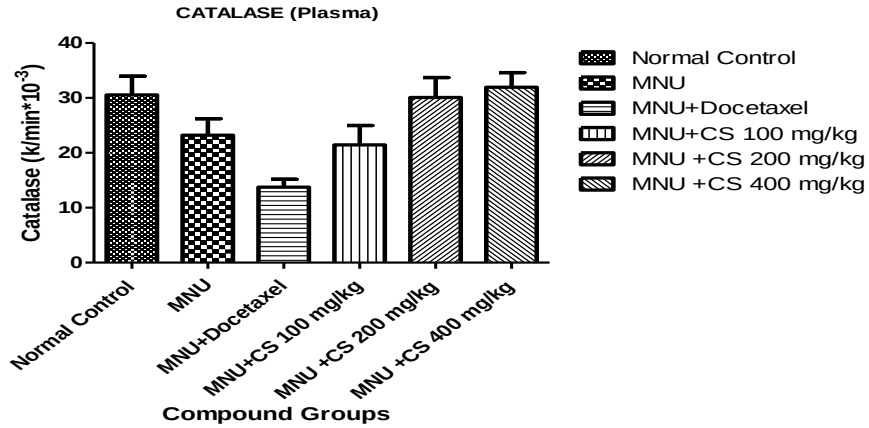



**Figure 26: Effect of CS on Catalase levels in Plasma**

**Table 17: Catalase Assay-Plasma**

| Compound Groups | Catalase(k/min*$10^{-3}$) |
|---|---|
| Normal Control | 30.56±6.76 |
| MNU | 23.22 ± 6.00 |
| MNU+Docetaxel | 13.74±2.85*** |
| MNU+CS 100 mg/kg | 21.44± 7.08 |
| MNU +CS 200 mg/kg | 30.09±7.27** |
| MNU +CS 400 mg/kg | 31.93±5.32** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on Catalase levels in Stomach:**

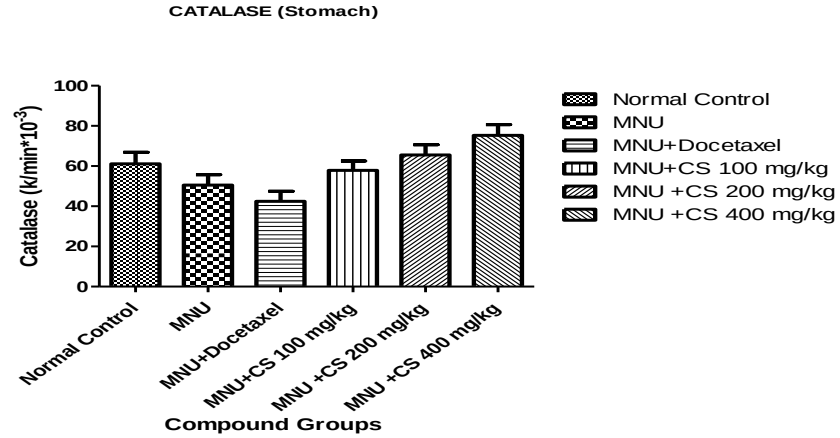



**Figure 27: Effect of CS on Catalase levels in Stomach**

**Table 18: Catalase Assay-Stomach**

| Compound Groups | Catalase(k/min*$10^{-3}$) |
|---|---|
| Normal Control | 61.15±13.99 |
| MNU | 50.57± 11.49 |
| MNU+Docetaxel | 42.45±12.26* |
| MNU+CS 100 mg/kg | 57.83± 10.52** |
| MNU +CS 200 mg/kg | 65.56±12.47** |
| MNU +CS 400 mg/kg | 75.26±13.12*** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <

0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+ docetaxel.

**Effect of CS on SOD levels:**
**Effect of CS on SOD levels in Liver:**

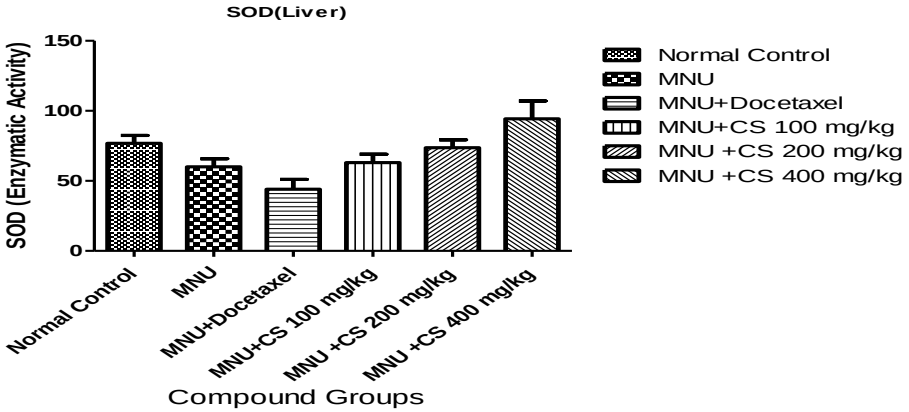



**Figure 28: Effect of CS on SOD levels in Liver**

**Table 19:SOD Assay-Liver**

| Compound Groups | SOD(% of Inhibition) |
|---|---|
| Normal Control | 76.806±13.92 |
| MNU | 60.018± 14.14 |
| MNU+Docetaxel | 44.02±15.64** |
| MNU+CS 100 mg/kg | 63± 13.6 |
| MNU +CS 200 mg/kg | 73.665±13.73** |
| MNU +CS 400 mg/kg | 94.26±31.21*** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on SOD levels in Plasma:**

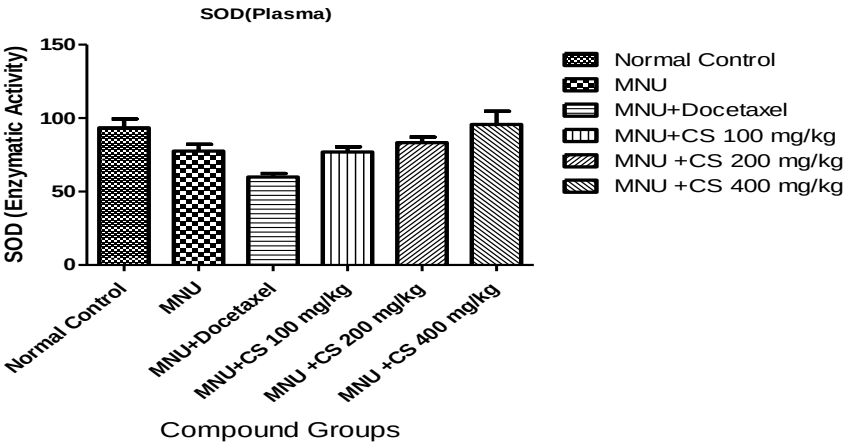



**Figure 29: Effect of CS on SOD levels in Plasma**

**Table 20: SOD Assay-Plasma**

| Compound Groups | SOD(% of Inhibition) |
|---|---|
| Normal Control | 93.41±11.99 |
| MNU | 77.57± 9.33 |
| MNU+Docetaxel | 59.97±4** |
| MNU+CS 100 mg/kg | 76.9825± 6.79 |

| | |
|---|---|
| MNU +CS 200 mg/kg | 83.3075±7.38* |
| MNU +CS 400 mg/kg | 95.6375±8.08** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+ docetaxel.

**Effect of CS on SOD levels in Stomach:**

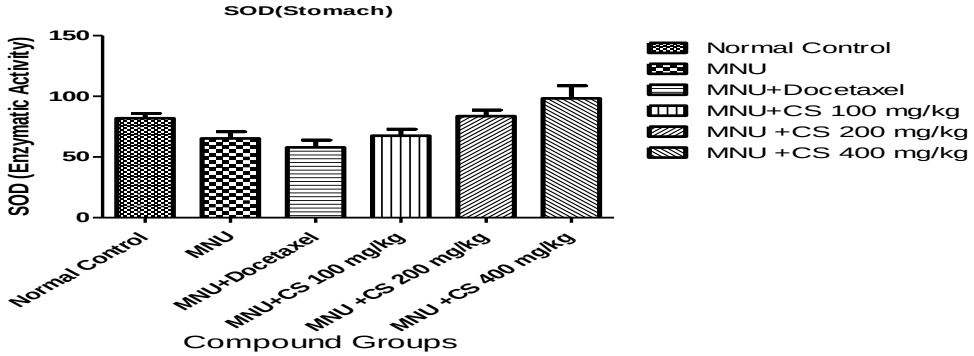



**Figure 30: Effect of CS on SOD levels in Stomach**

**Table 21: SOD Assay-Stomach**

| Compound Groups | SOD(% of Inhibition) |
|---|---|
| Normal Control | 81.96 ±9.89 |
| MNU | 65.31± 12.33 |
| MNU+Docetaxel | 57.9± 14.9* |
| MNU+CS 100 mg/kg | 67.6± 11.83 |
| MNU +CS 200 mg/kg | 83.71±12.28** |
| MNU +CS 400 mg/kg | 98.31±15.36*** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on ROS levels:**
**Effect of CS on ROS levels in Liver:**

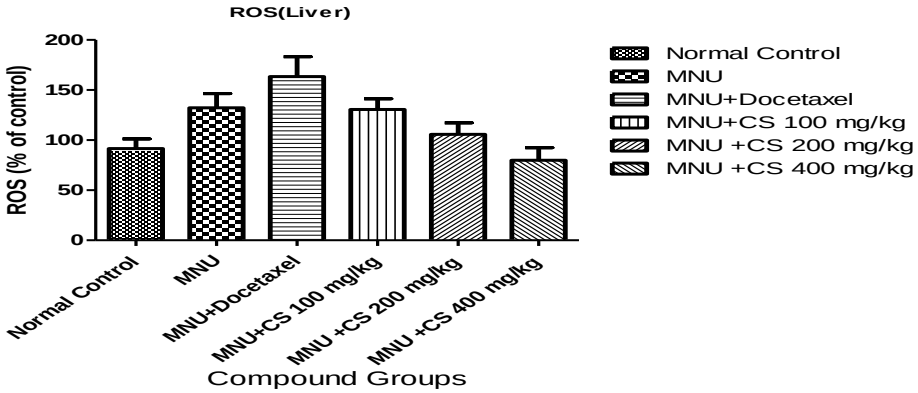



**Figure 31: Effect of CS on ROS levels in Liver**

**Table 22: ROS Assay-Liver**

| Compound Groups | ROS(% of Control) |
|---|---|
| Normal Control | 91.52 ±3.53 |
| MNU | 132.29± 1.65 |
| MNU+Docetaxel | 163.91± 4.54** |
| MNU+Docetaxel+CS | 103.98 ± 3.86* |
| MNU +CS | 105.8±2.94** |
| CS | 79.96±3.59*** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+ docetaxel.

**Effect of CS on ROS levels in Stomach:**

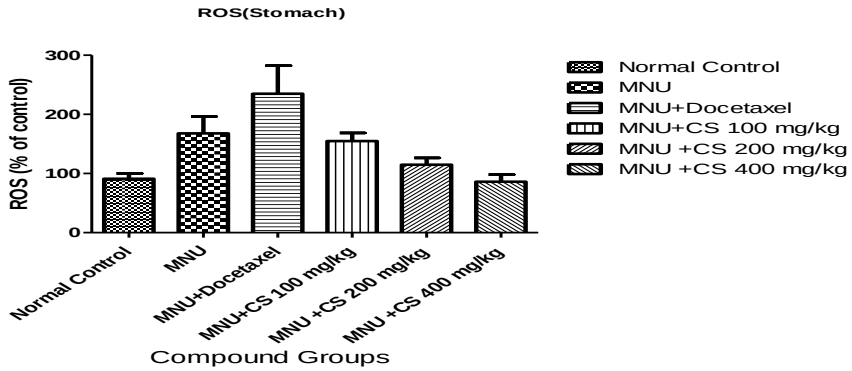



**Figure 32: Effect of CS on ROS levels in Stomach**

The figure shows the ROS values mentioned as Mean ± S.E.M (n=6). *Represents significant statistical difference (P<0.05) when compared with MNU+Docetaxel vs CS. **indicates significant statistical difference (P<0.01) when compared with MNU+DocetaxelvsCS and Control vs.MNU+Docetaxelgroups.

**Table 23:ROS Assay-Stomach**

| Compound Groups | ROS(% of Control) |
|---|---|
| Normal Control | 91. ±2.076 |
| MNU | 167.98± 3.44 |
| MNU+Docetaxel | 234.91± 6.44*** |
| MNU+CS 100 mg/kg | 154.98 ± 3.65 |
| MNU +CS 200 mg/kg | 114.8±8.07* |
| MNU +CS 400 mg/kg | 86.1±9.62*** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on Organ weights:**

**Effect of CS on Organ weights of Liver:**

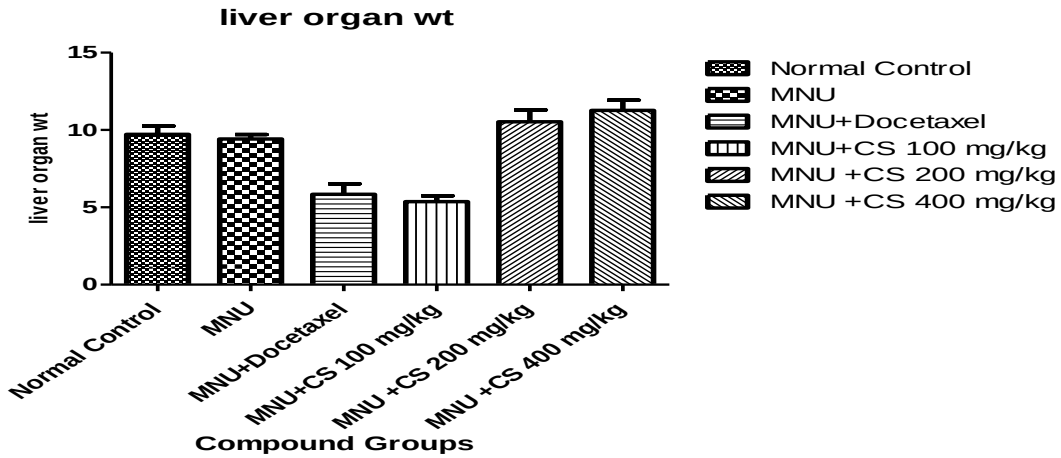



**Figure 33: Effect of CS on Organ weights of Liver**

**Table 24: Effect of CS on Organ weights of Liver**

| Compound Groups | Liver organ weight (grams) |
|---|---|
| Normal Control | 9.73 ±1.32 |
| MNU | 9.41± 0.69 |
| MNU+Docetaxel | 5.85± 1.62** |
| MNU+CS 100 mg/kg | 5.36 ± 0.93 |
| MNU +CS 200 mg/kg | 10.58±1.85* |
| MNU +CS 400 mg/kg | 11.26±1.64** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

**Effect of CS on Organ weights of Stomach:**

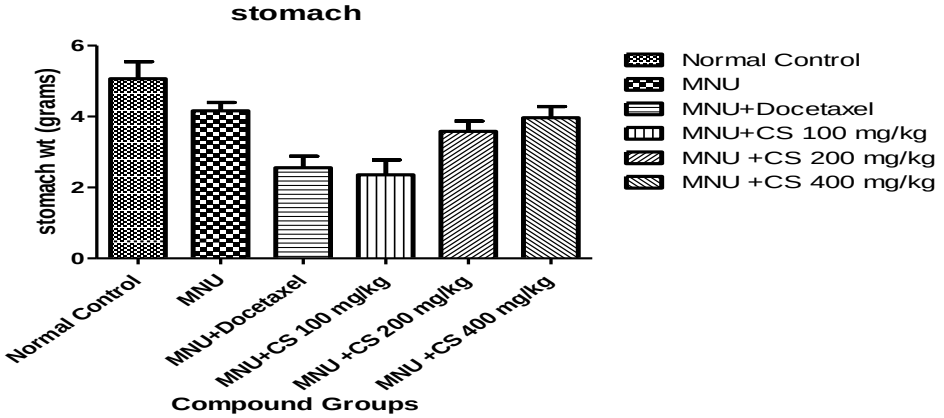



**Figure 34: Effect of CS on Organ weights of Stomach**

**Table 25: Effect of CS on Organ weights of Stomach**

| Compound Groups | Stomach organ weight (grams) |
|---|---|
| Normal Control | 5.06 ±1.17 |
| MNU | 4.16± 0.52 |
| MNU+Docetaxel | 2.56± 0.72** |
| MNU+CS 100 mg/kg | 2.36 ± 0.93* |
| MNU +CS 200 mg/kg | 3.58±0.7** |
| MNU +CS 400 mg/kg | 3.96±0.77** |

Values are mean ± SD of three replicates (n=6). Where a represents #P <0.001 as compared with control and *P < 0.05; **P < 0.01; and ***P < 0.001 in comparison with MNU+docetaxel.

## DISCUSSION

The conducted study confirmed that oral gavage of MNU- and s-NaCl-induced a 100% cancer incidence in rats. The histological outcomes showed that CS could attenuate the gastric carcinogenesis induced through MNU and s-NaCl in rats. CS has been implicated as having a potentially advantageous impact on several chronic ailments. Evidence from epidemiological and traditional data supports the aim pending the chemopreventive role of CS. In this study, a significant decrease in average body weight was observed in the cancer-induced and docetaxel-treated rats. The subsequent increase in body weight upon administration of CS could be because of the protective efficacy of the CS. CS may possess an excellent antitumor potential and antioxidant benefit, which might improve rats' health and digestive function. Subsequently, the increase in food intake might have facilitated the rise in body weight. Docetaxel is one of the best first-line drugs for the treatment of gastric cancer. The mechanisms responsible for Docetaxel-induced hepatotoxicity have not been wholly understood yet one possibility is Docetaxel has a direct toxic activity on hepatocytes, accounting for the rate of recurrence of serum enzyme rise during therapy, chiefly with higher doses.

**Catalase** is broadly dispersed in all tissues and catalyzes the breakdown of $H_2O_2$. The source of $H_2O_2$ is chiefly SOD-mediated dismutation of SOD radicals produced using systems of enzymatic and non-enzymatic. Quite a lot of reports have mentioned declined activities of SOD and catalase in a variety of carcinogenic surroundings. The observed demur in SOD and catalase activities in our investigation might be since the amplify in circulating lipid peroxides reportedly result in the build-up of superoxide anions that are competent of traversing membranes causing detrimental effects at sites further than the tumor . In this investigation, we evaluated the oxidant and antioxidant parameters of blood, liver and gastric tissues in gastric cancer rats. Significantly augmented MDA levels and lessened antioxidant enzyme activities were detected in gastric cancer rats. This implicates that there may be a relation between gastric cancer and gastric oxidant and antioxidant parameters, the administration of CS at 25 mg/kg doses lessened the level of oxidant parameters (MDA) and augmented blood, gastric and liver antioxidant parameters (SOD, CAT and GSH-Px) significantly. CAT, SOD and other antioxidants are endogenous factors that lessen the toxic role of ROS. So, we can hypothesize that CS may reduce oxidative injury of gastric cancer rats to a degree through

invigorating antioxidant enzyme actions. **The ROS levels** were diminished with the treatment of CS. Healthy tissue cells have a lot of mechanisms that be able to prevent the harmful effects of ROS or be able to repair existing damage—1) enzymes and 2)melatonin, glutathione, and antioxidant vitamins, avert tissue damage through preserve ROS at physiologic amounts in cells.

**Summary and conclusion:**

In summing up, the current study shows, Calycophyllum spruceanum extract treatment attenuated gastric cancer induction by MNU and significant positive relation with therapies with medicinal plant extracts treated groups by not causing side effects like liver damage by keeping normal SGPT, SGOT values and improving the status of gastric cancer compared to Docetaxel treated group. Further studies are warranted to establish the optimum effective dose of these phytochemical compounds, Calycophyllum spruceanum extract in inhibiting cancer in humans.

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