Inventum Biologicum: *An International Journal of Biological Research*
2025, Vol. 5, Issue 4, pp. 28-49

Inventum Biologicum

Journal homepage: www.journals.worldbiologica.com/ib

Research paper

Protective Effects of β-carotene Against Sodium Dodecyl Sulfate Induced Stress in Male Swiss Albino Mice (*Mus musculus*)

A Multi-Index Study of Histopathological, Hematological, Cytogenetic and Biochemical Parameters

Biswadip Sanfui [a], **Debasmita Ghosh** [a], **Sromon Dutta** [a], **Ritika Sarkar** [a], **Sandip Kumar Mandal** [a], **Sarmistha Banik** [a], **Sujit Kumar Bhowal** [a] *

[a] *Molecular Cell Biology Lab, Department of Zoology, Maulana Azad College, 8, Rafi Ahmed Kidwai Road, Taltala, Kolkata, West Bengal-700013, India*

ARTICLE INFO

Keywords

Sodium dodecyl sulphate
Cytotoxicity
β-carotene
Sperm head anomaly
ROS
Catalase activity
Micronuclei
Chromosome aberration
Mitotic index

DOI
10.5281/ib-2180825

***Corresponding author**
Sujit Kumar Bhowal

✉ **Email**
sujit.sb@gmail.com

ABSTRACT

Sodium Dodecyl Sulfate (SDS) is a very common anionic surfactant, which is extensively used in daily life. SDS finds applications in the food industry, cosmetics industry and other healthcare products. Regular exposure to SDS in day-to-day life may be hazardous due to its potential cytotoxic nature. Hazardous effects can be seen when animals are exposed to it via oral and dermal routes. There have been four experimental groups, Gr. I (negative control), Gr. II (200mg/ Kg for 7 consecutive days β-carotene positive control), Gr. III (0.06 g/Kg body weight SDS control) and Gr. IV β-carotene for 7 consecutive days and then β-carotene for another 7 consecutive days along with SDS). It has been shown that organs like liver, pancreas and testis of animals of Gr. III are adversely affected and disruption of hepatocytes, pancreatic cells and testicular cells have been observed. SDS adversely affects haematological parameters by lowering haemoglobin level and erythrocyte count, and increasing leukocyte count. From the biochemical point of view SDS has been found to increase Reactive Oxygen Species (ROS), lower tissue protein content and catalase activity. In addition to testicular histopathological effects, SDS has lowered sperm count, sperm motility, and increased sperm head anomalies. Apart from these, the cytotoxicity of SDS is recorded concerning cell viability, mitotic index, micronuclei as well as chromosome aberrations. β-carotene has been known as a potent antioxidant and it might have some role on amelioration of the harmful effects of SDS. With this hypothesis and consideration, SDS-induced mice have been exposed to β-carotene (Gr. IV) to obtain the amelioration effect on toxicity that involve oxidative mechanisms. From the results of our investigation, it is evident that SDS causes wide ranging toxic effects on the health of male Swiss albino mice, affecting growth, organ function, blood profile, sperm quality, genetic integrity and oxidative balance. These adverse effects appear to be closely linked with oxidative stress and cellular damage. β-carotene, on the other hand, is able to reduce or prevent many of these SDS-induced effects. It may be thought that antioxidants like β-carotene might work in the pathway that neutralizes ROS generated by SDS exposure, thereby reducing oxidative stress, and preventing cellular damage.

1. Introduction

Sodium Dodecyl Sulfate (SDS) is used as a part of household goods of daily use. Having a detergent property as an anionic surfactant, it is widely found in cleaning or grooming products, toothpaste, soaps and shampoos. It is also used industrially for softening of leather, wool cleaning and has an important role in the cleaning products used for automobile washes. SDS is also extensively used in laboratories for the polyacrylamide gel electrophoresis and other uses of cryopreservatives (Kumar *et al.*, 2014). SDS is an alcohol detergent derived from Alcohol Sulfates, also known as Sodium Lauryl Sulfate is represented by molecular formula $C_{12}H_{25}NaO_4S$ with molecular weight of 288.38 g mol^{-1}(Ashok *et al.*, 2011).

Evaluating the destructive effects of SDS on the DNA, RNA, proteins and enzymes, physiology, cytology, as well as the metabolism of plants, animals, and even mammals is important. The area of environmental mutagenesis, in particular, requires further investigation to assess the many noxious wastes of so-called progressive civilization, which are bestowed upon nature every day. Oral consumption of 0.5–5.0 g/kg body-weight of SDS can be fatal or severely harm human health (Bidevkina *et al.*, 2020). Direct contact to SDS (≤20%) may cause mild skin irritation, repeated exposure can lead to dermatitis like redness, swelling and blistering (Agner, 1991). Exposure to SDS has been shown to increase production rate of ROS (Reactive Oxygen Species) & alteration of the antioxidant defense mechanism, resulting in oxidative stress (Siems *et al.*, 2002, Svegliati *et al.*, 2001).

β-carotene, a potent antioxidant, might be able to ameliorate the harmful effects of SDS. SDS-induced toxicity involves oxidative mechanisms; it may be thought that antioxidants like β-carotene might work in the pathway that involves the antioxidant neutralizing ROS generated by SDS exposure, thereby reducing oxidative stress and preventing cellular damage (Josson & Sabina, 2020, Lawlor *et al.*, 1995). These properties help to prevent cellular damage and inhibit the development of cancer. Though, its high doses may have adverse effects.

2. Aim and Objectives

This study aims to investigate the effects of SDS exposure and ameliorating upshot of β-carotene on morphometric, histological, hematological, cytologic-al, viability of bone marrow cells and biochemical parameters pertaining to spectrophotometric quantification of whole tissue protein, estimation of tissue nitrite (NO_2^-) content and catalase activity of Swiss albino mice have been investigated.

3. Methodology

3.1 Experimental Animals

In the present study, healthy inbred strain of male Swiss Albino mice (*Mus musculus*), reared and maintained in the animal house of Department of Zoology (under the supervision of The Animal Welfare Committee), Maulana Azad College, served as materials. Mice were provided with food and water *ad libitum.* The food was generally made up of flour, gram and powdered milk which contained no animal protein supplementation, unless mentioned otherwise. The experimental protocols were in accordance with the guidelines laid down by the Animal Welfare Committee, Maulana Azad College.

3.2 Experimental Protocol

3.2.1 Dose Selection

In the present study, evaluation of the effects on various parameters by the treatment with SDS and β-carotene as a dietary supplement, dose selection and feeding method used by several previous workers has been adopted. Sodium dodecyl Sulfate (SDS) is used as the treatment agent. Concentrations of SDS were determined or calculated on the basis of known data of SDS toxicity. In oral administration method, lethal dose (LD_{50}) of SDS for mice was assumed to be 0.12 g/kg body weight (Irizarry *et al.,* 2021). The chemical used is reagent grade and was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

After proper calculations, 50% of LD_{50} of SDS was prepared, then dissolved in distilled water to prepare stock solution [0.25 g of sodium dodecyl Sulfate (50% of LD_{50}) was dissolved in 50 ml distilled water]. Force feeding (oral gavage method) was done at the dose of 1.2 ml /100g body weight on daily basis.

In the present study, to observe amelioration effects of β-carotene on SDS treated mice, β-carotene powder was used from capsules. The dosage of β-carotene was determined from the method used by several workers (Salvadori *et al*., 1992). 200mg/kg was assumed to be the dose of β-carotene for mice. β-carotene capsules were purchased from Biotrex Nutraceuticals, Gujarat, India.

After accurate calculations, dosage stock was prepared (6 mg of β-carotene, mixed with 15g flour, 5g milk powder and 100µl olive oil). Feeding was one at the dose of 24 mg/100g body weight on daily basis.

3.2.2 Experimental Animals: Control And Treatment Series

Healthy male mice (Four groups, each having 5 mice) weighing between 24 and 26 grams were chosen for the present studies.

Negative control / Normal: Five male mice fed with normal diet were maintained during the study and were sacrificed after 7 days for studying the normal values or parameters. These male mice served as negative control.

Positive control / β-carotene fed series: Five male mice fed with diet mixed with β-carotene powder (200mg/ kg body weight) once a day (12:00 o'clock) on a daily basis for 7 consecutive days and then sacrificed for studying the effects of β-carotene. These male mice served as positive control.

Sodium Dodecyl Sulfate (50% of LD_{50}) fed series: Five male mice fed with normal diet were gavaged with 50% of LD_{50}Sodium Dodecyl Sulfate (0.06g/kg body weight) once a day (11:00 am) on a daily basis for 7 consecutive days. Dosage was calculated before, according to their body weight, which was recorded regularly.

Both SDS and β-carotene fed mice: Five male mice were fed with 50% of LD_{50} SDS (0.06g/kg body weight) once a day (11:00 am) on a daily basis for 7 consecutive days. They were fed with β-carotene (200mg/ kg body weight), 1 hour after SDS treatment on a daily basis for same 7 consecutive days, once a day at 12:00 o'clock. Dosage was calculated before, according to its body weight.

3.3 Selection of Tissues

Study of histopathological study: For studying the histopathological study, following body tissues (or organs), were considered:

- Liver
- Pancreas
- Testis

Study of haematological study: For studying haematological study, blood is used.

Study of cytogenetic study: For study of cytogenetic study, sperm threads are collected from vas deferens and epididymis (especially from cauda epididymis) and bone marrow cells are collected from femoral bone of mice.

Study of biochemical parameters: For studying biochemical parameters, liver and testis tissue are used.

3.4 Statistical Analysis

All values represented in the experiments are considered as mean ± standard error (S.E.) of five replicas. The mean values were compared between control and treated groups for evaluation of any significant changes by One-way or Univariate Analysis of Variance test (ANOVA) and Tukey HSD Test. $P < 0.0001$, $P<0.001$ or $P<0.05$ are considered statistically significant as the case may be.

3.5 Cytogenetic Study

For cytogenetic study like cell viability, mitotic indices, chromosomal aberration and micronuclei test, cells from bone marrow were processed while for sperm head anomaly and sperm count epididymis and vas deferens were processed

3.5.1 Study of Bone Marrow Cell Viability

Study of cell viability from bone marrow cell harvested from mice is done with Trypan blue technique (Tennant *et al.,* 1964).

Collection of Bone Marrow Cells: Marrow of the femur was flushed in ice cold PBS solution, and brought into suspension by repeated flushing in and out of a pipette with rubber tip.

Preparation of Cell Mixture: The cell suspension was centrifuged for 5 min at 15000 rpm and the supernatant was discarded. The cell pellets were resuspended in 1 mL PBS. 0.4% trypan blue and cell suspension were mixed and the mixture was incubated for 3 min at room temperature. Mixing was performed in a well of a microtiter plate or a small plastic tube using 10-20 µL cell suspension and trypan blue. A drop of the trypan blue/cell mixture was applied to a hemocytometer. The hemocytometer was placed on the stage of a microscope and the cells were focused. The unstained (viable) and stained (nonviable) cells were counted separately in the hemocytometer.

3.5.2 Study of Mitotic Index and Chromosomal Aberrations

Collection of bone marrow cells: Mice were injected intraperitoneally with 0.03% colchichine (1ml per 100 gram body weight), at all fixation intervals 1 hour and 15 minutes before sacrifice. Marrow of the femur was flushed in 1% sodium citrate (Merck, India, MJ6M562587) (hypotonic)solution, and brought into suspension by repeated flushing in and out of a pipette with rubber tip and incubated for 7-10 minutes at 37°C.The materials were centrifuged for 6-8 minutes at 7000g and the supernatant was discarded.

Preparation of slides: Bone marrow cells of control and treated series were smeared uniformly on clean grease free glass slides. Semidried slides were dipped in 90% ethyl alcohol briefly and allowed to air-dry. Air-dried slides were stained for 5 minutes in May-Grunwald-Giemsa stain as per the routine procedure by mixing1 part of stock solution and 1 part of Milli-Q water. The slides were rinsed in double distilled water and finally stained in diluted Giemsa (1 part stock Giemsa and 10 part double distilled water).

3.5.3 Study of Erythrocyte Micronuclei

Collection of bone marrow cells: The abdomen is cut open to dissect out the femoral bone of either site. The bone marrow is flushed out by glass syringe in 4 to 5 ml of 1% sodium citrate solution. It is thoroughly flushed in hypotonic solution with a Pasteur pipette. The suspension is then centrifuged at 2000 rpm for 5-6 minutes.

Preparation of slides: The suspension of bone marrow cells in 1.0% sodium citrate solution is smeared in grease-free glass slides. The smeared cells are air-dried, briefly fixed in methanol and stained for three minutes in stock May-Grünwald stain followed by staining again with diluted May-Grünwald stain for five minutes. The slides are rinsed in distilled water and finally stained in diluted Giemsa's stain for about fifteen minutes. Post-staining the slide is rinsed in distilled water and air-dried, cleaned in xylene and mounted in DPX.

3.5.4 Study of Sperm Count and Sperm Head Anomaly

Collection of sperm threads/ sperm trails: The vas deferens was separately dissected and squeezed with the help of watchmaker forceps to squeeze out the sperm thread into 0.89% saline prepared freshly.

Preparation of haemocytometer for Sperm Count: The sperm count was done by using haemocytometer (Sokol *et al.,* 2000). Sperm is diluted with 0.89% saline. 10-15 µl of the diluted sperm is placed under the cover slip on each side of the hemocytometer. The hemocytometer is placed on a microscope. The hemocytometer is viewed with a 40X objective. Concentration/ml = (Dilution Factor)(Count in 5 squares)(0.05× 10^6). By convention, sperm concentration is usually expressed in terms of sperm × 10^6/ml.

Preparation of slides for Sperm Head Anomaly: The vas deferens and epididymis of each side of the male mice was dissected out and taken separately into 5 ml of 0.89% normal saline. It was made free of fats, vas deferens and epididymis and other tissues. The inner content of each side of the vas deferens and epididymis was taken out in normal saline and the material was thoroughly shaken to suspend the sperm in saline solution. The sperm suspension was filtered through silken cloth to remove the debris and the filtrate was collected in a graduated tube, more saline was added to make the volume 10 ml. The sperm suspension thus collected, was loaded on a clean slide over which 0.5ml methanol was added. The material was allowed to dry. A drop of diluted Giemsa stock solution (1:10) was put on the material. The material was covered with a cover glass and sealed temporarily for observation as per the routine procedure (Wyrobek *et al.,* 1984).

3.6 Biochemical Study

3.6.1 Collection of tissue

Liver and testes tissue was weighed with the help of a digital Petit balance (BSA224S-CW) and homogenized with PBS and supernatant was collected after centrifugation. For study of protein content and catalase enzyme activity the liver tissue was homogenized. For study of nitrite concentration (ROS) liver tissue and testis tissue was homogenized.

3.6.2 Study of Nitrite (ROS) content (Quantitative)

Estimation of NO_2^- in the protein lysate from tissue samples was evaluated by the measurement of nitrite (NO_2^-), the stable product of decomposition, employing the Griess reaction (Green *et al., 1982*).

3.6.3 Study of Catalase Enzyme Activity (Quantitative)

The catalase activity was determined by simple spectrophotometric assay (Hadwan, 2018). To perform the assay the following reagents were collected or prepared and conventional protocols were followed.

Reagent	Sample Tube	Standard Tube	Blank Tube
Catalase Sample	500 µL	-	-
Distilled Water	-	500 µL	1500 µL
Hydrogen Peroxide (10 mM)	1000 µL	1000 µL	-

Vortexing and incubation was done at 37°C for 2 minutes. 6000 µL Working Solution was added to each tube. Vortexing was again done for 5 seconds. Incubation was done at room temperature in the dark for 10 minutes. Absorbance was measured at 440 nm against the blank.

3.6.4 Study of Tissue protein Content (Quantitative)

Total tissue protein content was determined by Lowry method (Lowry *et al.,* 1951). To perform Lowry method to estimate the concentration of protein, protein reagent was prepared and conventional protocols were followed.

3.7 Haematological Study

3.7.1 Collection of Blood and Storage

Approximately 1.5 ml of blood from mice heart was collected by the process of cardiac puncture after the animal was anaesthetized using Chloroform and subsequently by using a 2 ml Dispovan Syringe with needle. The blood was collected in 2 ml eppendorfs, previously coated with EDTA to prevent coagulation. The collected blood was preserved for future uses in a refrigerator.

3.7.2 Study of Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC) from Fresh Blood Sample

The total number of RBC and WBC per mm^3 of blood is determined by diluting a known volume of blood with a fluid which is isotonic with blood and prevents its coagulation. The blood cells are then counted in known volume of dilute blood in counting chamber (haemocytometer) and the number of RBC or WBC per mm^3 of the undiluted blood is then determined by calculation.

3.7.3 Study of Differential Count of Leukocytes

Count of different leukocytes (Banerjee, 2021) was calculated after preparation of blood film and staining the same with Leishman's Stain.

3.7.4 Study of Haemoglobin concentration

The haemoglobin (Hb) of the blood is converted into acid-haematin by adding N/10 HCl and the content is determined by matching the solution against a non-fading coloured glass.

3.8 Histological Study

For histopathological observation by light microscopy, histology slides of liver, testes and pancreas were prepared. Sections were counterstained with hematoxylin-eosin using standard protocol and mounted with a coverslip in DPX. Histopathological observations were done with a bright field microscope (Magnus, India) and photographs were captured using Zeiss AxioCam ICc1 and Zen software (Zen2 lite).

4. Results

4.1 Cytogenetic study

4.1.1 Study of Bone Marrow Cell Viability

The number of viable cells of control mice was **3.154 x 10^6** /mL of aliquot. In case of positive control of β-carotene the number of viable cells was **3.178 x 10^6** /mL of aliquot. The number of viable cells was found to be considerably reduced in SDS fed mice being **0.21 x 10^6** /mL of aliquot. In the mice fed with β- carotene and SDS the number of viable cells was **1.734 x 10^6** /mL of aliquot. The statistical significances are denoted in the following table.

Table 1 Cell Viability Count

Treatment series	Viable Cells Per mL of Aliquot (x10^6/ mL)
Control mice	3.154*±0.05
β- carotene treated mice	3.178*± 0.031
SDS treated mice	0.21*± 0.003
β- carotene + SDS treated mice	1.734* ± 0.005

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.001]

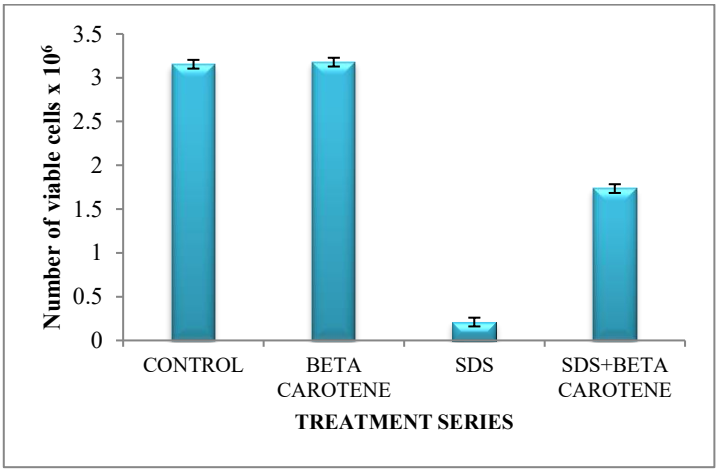


Fig. 1 Graphical representation of Viable cell count

4.1.2 Mitotic Index

In the control group, there were 130.6 dividing cells and 140.2 non-dividing cells, resulting in a mitotic index of 48.23%. Treatment with β-carotene led to a marked increase in the number of dividing cells (319.2) and non-dividing cells (227.4). The mitotic index also rose to 58.39%, higher than control. In contrast, exposure to SDS resulted in a sharp decline in the number of dividing cells (101) with a substantial rise in non-dividing cells (441.8). The corresponding mitotic index dropped drastically to 18.61%, indicating severe inhibition of cell division. When SDS and β-carotene were administered together, the number of dividing cells increased to 126, while non-dividing cells decreased to 363.4, resulting in a mitotic index of 25.75%. Although this value is significantly lower than in the control and β-carotene-only groups, it is markedly higher than in the SDS-only group. This indicates that β-carotene provides partial protection against the inhibitory effects of SDS on cell division.

Table 2 Mitotic Index

	Dividing Cells	Non-Dividing Cells	Mitotic Index (%)
Control	130.6 ± 3.44	140.2 ± 3.84	48.23 ± 0.52
Beta Carotene	319.2 ± 4.85	227.4 ± 4.17	58.39 ± 0.81
SDS	101 ± 5.36	441.8 ± 11.28	18.61 ± 0.91
SDS+Beta Carotene	126 ± 4.66	363.4 ± 5.97	25.75 ± 0.98

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.001]

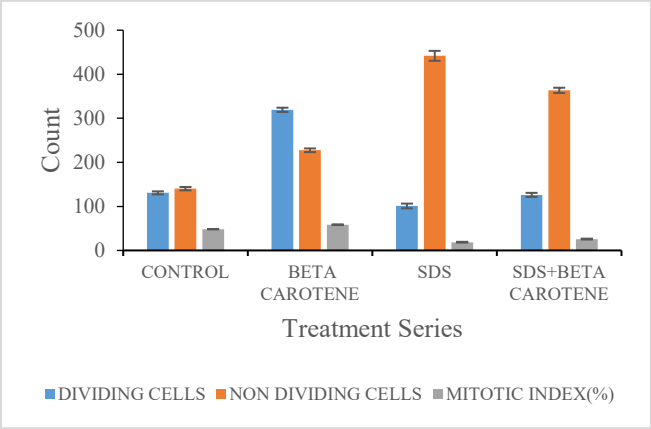


Fig. 2 Graphical representation of mitotic indices

4.1.3 Chromosomal Aberrations

In the control group, 71.6 out of 100 cells were normal and 28.4 were abnormal. In the group treated with β-carotene alone, the number of normal cells increased to 85, and the number of abnormal cells dropped to 15. In contrast, treatment with SDS caused a strong negative effect. Only 21.4 cells were normal, while 78.6 showed chromosomal abnormalities. However, when mice were treated with both SDS and β-carotene together, the number of normal cells rose to 61.4 and abnormal cells dropped to 38.6. On further observations in the control group, it is found that the numbers of specific changes like aneuploidy, polyploidy, strand breaks, stickiness, and fragments were moderate. The total number of chromosomal abnormalities was 36.6. In the β-carotene group, these numbers were lower in almost every category, and the total dropped to 21. In the SDS group, the number of abnormalities went up sharply. There were high counts of single-strand breaks (28.4), chromosomal stickiness (27.6), and fragments (21.8), along with increases in other damage types. The total reached 95.6. In the group treated with both SDS and β-carotene, the numbers of abnormalities were lower than with SDS alone. Single-strand breaks dropped to 10.4, stickiness to 14, and fragments to 10. Other categories also showed small reductions. The total number of chromosomal problems in this group was 46.6. Although this is still higher than the control group, it is much less than in the SDS-only group. This shows that β-carotene was able to reduce some of the chromosomal damage caused by SDS.

Table 3 Normal and Aberrant Cell Count

	Control	β- Carotene	SDS	SDS + β- Carotene
Normal Cells	71.6 ± 1.208	85 ± 0.707	21.4 ± 0.509	61.4 ± 0.509
Aberrant Cells	28.4 ± 1.208	15 ± 0.707	78.6 ± 0.509	38.6 ± 0.509

Table 4 Differential Chromosomal Aberration Study

	Control	β- Carotene	SDS	SDS + β- Carotene
Aneuploidy	5 ± 0.707*	2.2 ± 0.374*	4.6 ± 0.509	4.6 ± 0.509*
Polyploidy	4.6 ± 0.748*	2.2 ± 0.374*	8.6 ± 0.509	4.6 ± 0.509*
Single Strand Break	8.8 ± 0.663*	4 ± 0.707*	28.4 ± 0.400	10.4 ± 0.927*
Double Strand Break	2.4 ± 0.309*	1.8 ± 0.374*	4.6 ± 0.509	3 ± 0.707*
Stickiness	6.4 ± 0.509*	3.8 ± 0.374*	27.6 ± 0.509	14 ± 0.707*
Fragments	9.4 ± 0.509*	6 ± 0.707*	21.8 ± 0.735	10 ± 0.707*
Total Aberration Events	36.6 ± 1.631*	21 ± 0.583*	95.6 ± 0.509	46.6 ± 0.509*

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.001]

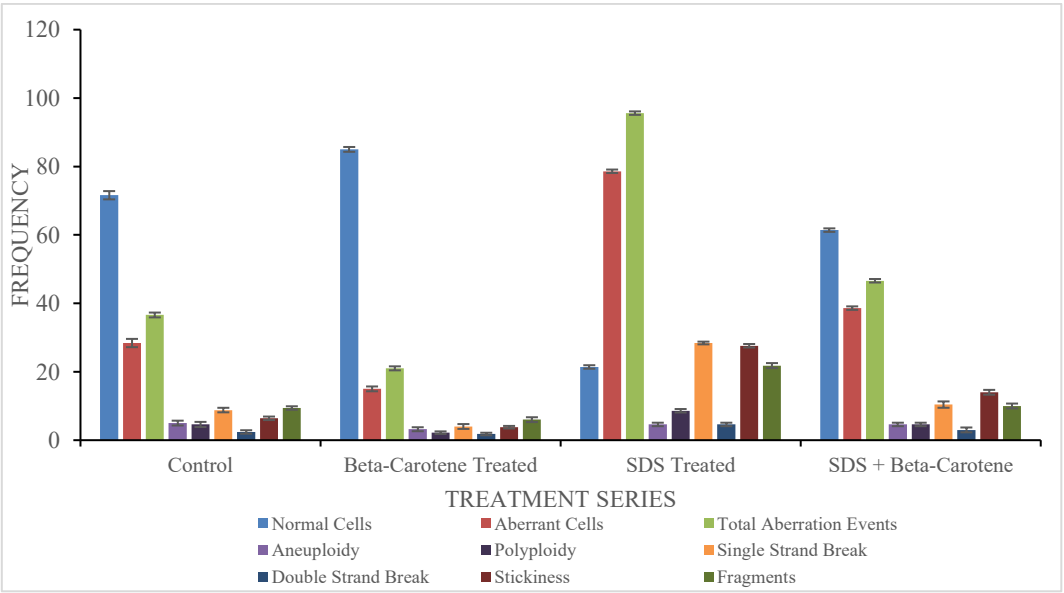


Fig. 3 Graphical representation of frequency of different types of aberrations in each of the four-treatment series

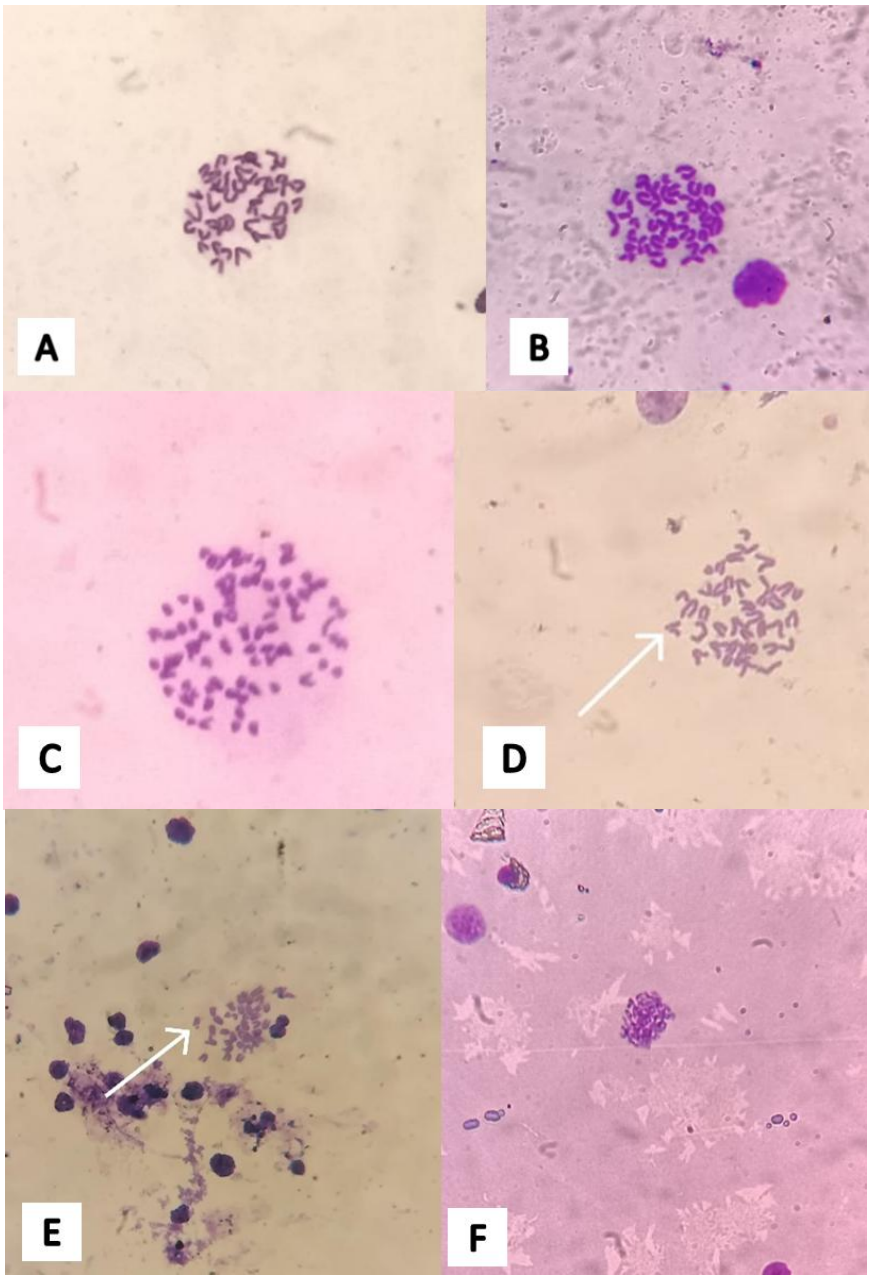


Fig. 4 Photographs of Normal Chromosomal Metaphase Plates [A, B], Polyploidy [C], Single Stranded Breakage in Chromosomal Plates [D, E], Stickiness [F] in Chromosomal Plate as harvested from bone marrow cells of mice after SDS treatment

4.1.4 Study of erythrocyte micronuclei

A study of approximately 600 erythrocyte cells per group was conducted to evaluate the effect of SDS and β-carotene on micronucleus formation. In the control group, the number of polychromatic erythrocyte (PCE) cells was 322.6, while the number of normochromatic erythrocyte (NCE) cells was 302.4. Among the PCEs, 26.4 contained micronuclei and 296.2 were without micronuclei. Similarly, among NCEs, 36.8 contained micronuclei and 265.6 were without micronuclei. In the group treated with β-carotene alone, the number of PCEs increased to 333.2, while NCEs were 300.8. Of these, 24 PCEs contained micronuclei and 309.2 were without micronuclei, whereas in NCEs 44.4 contained micronuclei and 256.4 were without micronuclei. In contrast, the SDS-treated group showed a reduction in erythropoietic activity. The number of PCEs decreased to 163.8, while NCEs increased to 456. Importantly, the number of PCEs with micronuclei increased markedly to 101.4, while only 62.4 PCEs were without micronuclei. Among NCEs, 341.2 contained micronuclei, with only 114.8 being without micronuclei. This reflects a strong genotoxic effect of SDS. In the group treated with SDS and β-carotene, partial protection was observed. The number of PCEs recovered to 274.2, while NCEs were 357. Of the PCEs, 66.2 contained micronuclei and 208 were without micronuclei. Among the NCEs, 116.8 contained micronuclei and 240.2 were without micronuclei.

Table 5 Micronuclei Test

	WMNPCE	MNPCE	PCE	WMNNCE	MNNCE	NCE
Control	296.2 ± 0.8*	26.4 ± 0.88*	322.6 ± 1.21*	265.6 ± 1.97*	36.8 ± 1.02*	302.4 ± 2.84
β-carotene	309.2 ± 1.6*	24 ± 1.52*	333.2 ± 2.38*	256.4 ± 1.78*	44.4 ± 1.57*	300.8 ± 3.11*
SDS	62.4 ± 0.82	101.4 ± 2.16*	163.8 ± 2.71*	114.8 ± 0.97*	341.2 ± 1.47	456 ± 1.31*
SDS + β-carotene	208 ± 1.23*	66.2 ± 1.72	274.2 ± 1.78	240.2 ± 2.23*	116.8 ± 0.67	357 ± 2.31*

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.001]

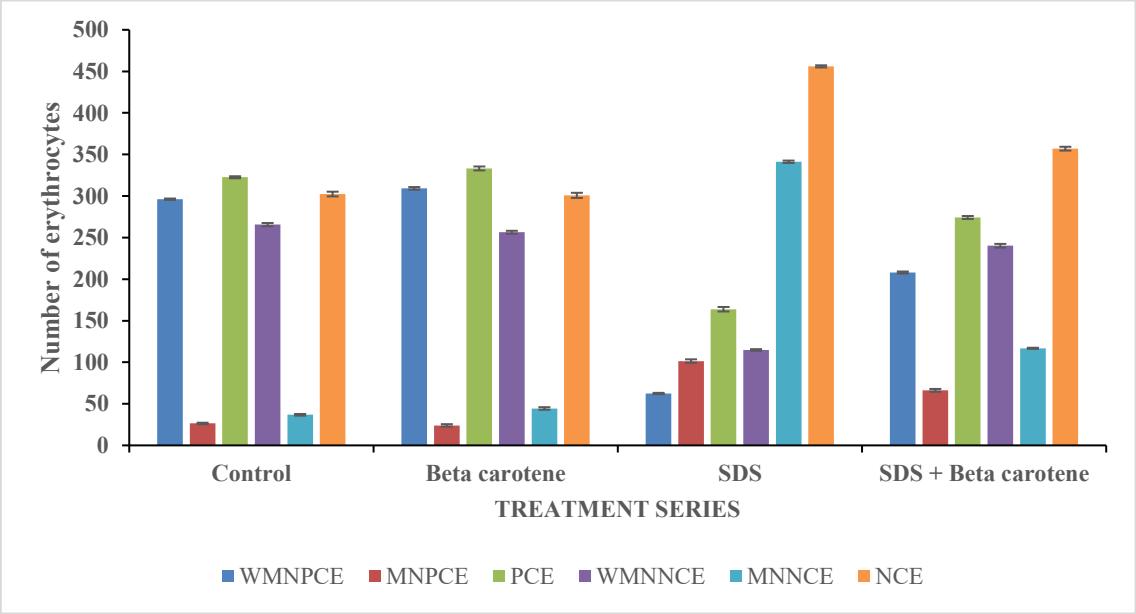


Fig. 5 Graphical Representation of Micronuclei Test

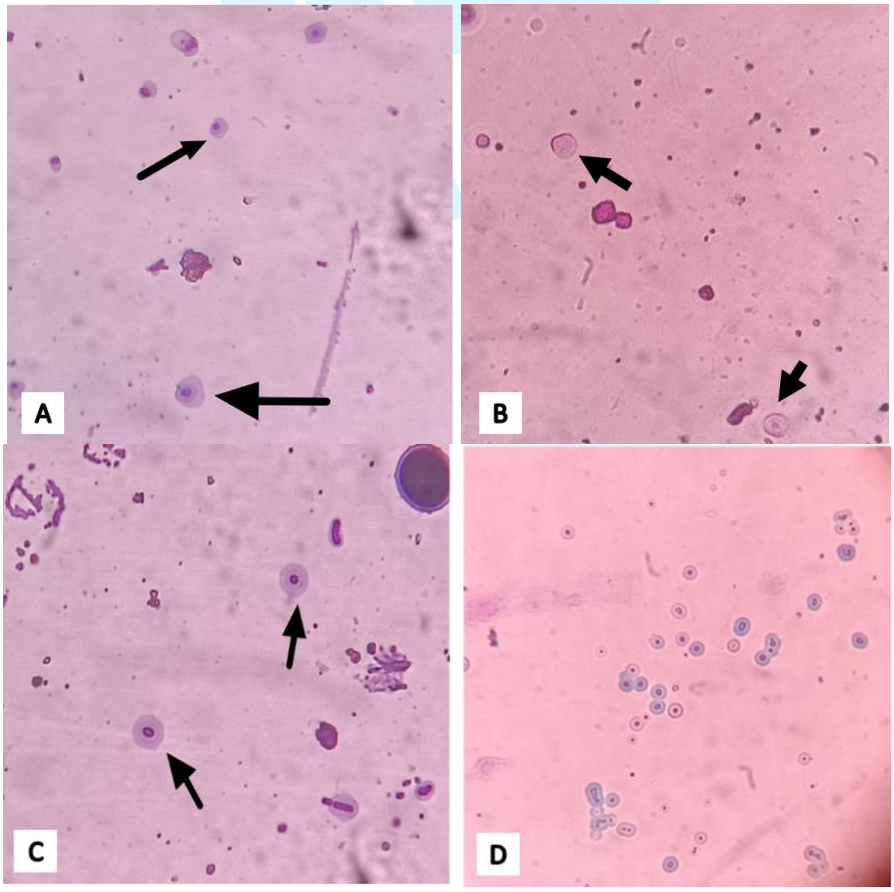


Fig. 6 Photographs of Micronucleus Tests Slides at 40X magnification of Magnus binocular microscope. [A] Normochromatic Erythrocyte Cells with Micronuclei [B] Polychromatic Erythrocyte Cells Normal (Centre) and Micronucleated (Right Corner) [C] Polychromatic Erythrocyte Cells with Micronuclei [D] Multinucleated Normochromatic and Polychromatic Erythrocyte Cells

4.1.5 Sperm Count

In control mice total sperm count was found to be **4.72×10^7/ml**. In β-carotene treated mice total sperm count was found to be **4.8×10^7/ml** demonstrating the anti-oxidative effect of β-carotene. In the SDS treated mice the total sperm count was reduced to **1.89×10^7 /ml**. In the mice treated with β- carotene and SDS the count increased to **3.96×10^7/ ml**. The summarized data of total sperm count in mice of different groups along with their statistical significances have been presented in following table.

Table 6 Sperm Count

Treatment series	Sperm Count/ ml
Control mice	$4.72 \pm 0.0938 \times 10^7$
β- carotene treated mice	$4.80^* \pm 0.0937 \times 10^7$
SDS treated mice	$1.89^* \pm 0.0775 \times 10^7$
β- carotene + SDS treated mice	$3.96^* \pm 0.0658 \times 10^7$

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.0001]

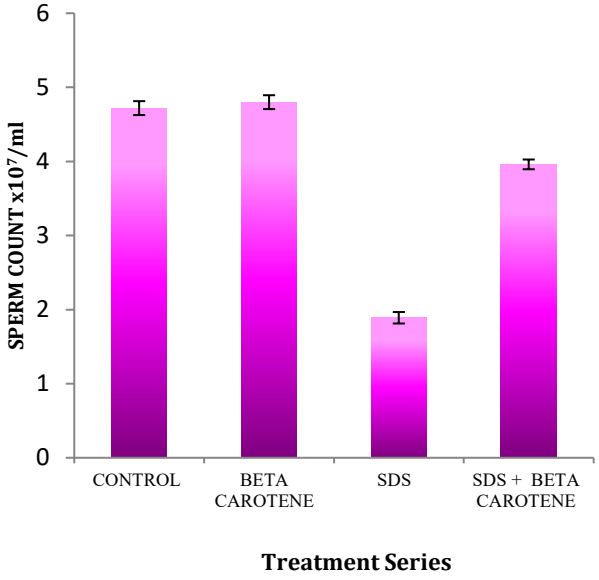


Fig. 7 Graphical representation of Sperm count / ml

4.1.6 Sperm Head Anomaly

In control mice **4.774%** sperm showed abnormal head morphology. Therefore, this could be taken as the baseline data on the incidence of abnormal sperm head as a result of background effect. In β-carotene treated mice **4.128%** sperm showed abnormal head morphology demonstrating the anti-oxidative effect of β-carotene. In the SDS treated mice the frequency was elevated to **52.816%**. In the mice treated with β- carotene and SDS the frequency was lowered to **11.088%**. The summarized data of sperm head anomaly (%) in mice of different groups have been presented in following table.

Table 7 Sperm Head Anomaly

Treatment series	Sperm Head Anomaly (%)
Control mice	4.774 ± 0.1598
β- carotene treated mice	4.128* ± 0.1255
SDS treated mice	52.816 ± 0.6073
β- carotene + SDS treated mice	11.088* ± 0.2381

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.001]

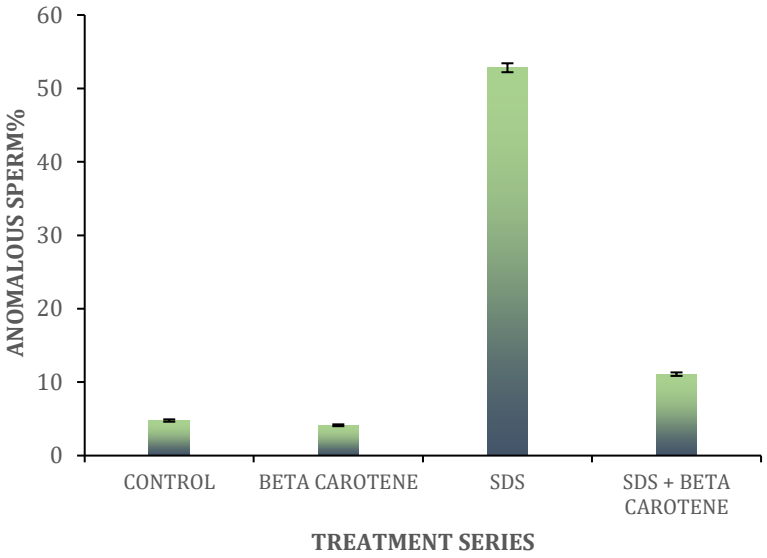


Fig. 8 Graphical representation of Sperm Head Anomaly (%)

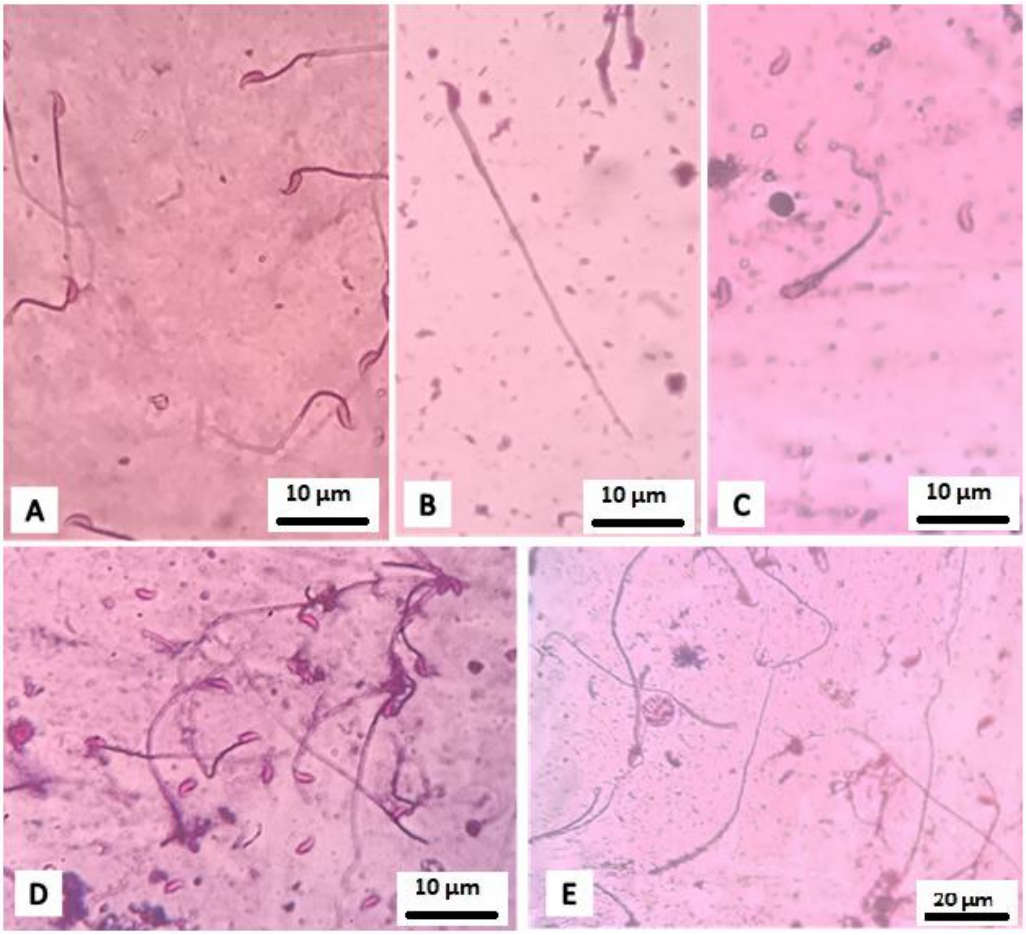


Fig. 9 Pictograph of sperm of mice showing [A,B] normal sperm as seen in the control group and β- carotene supplemented mice [C] sperm head anomaly (rosette shape of sperm head), [D,E] detached sperm heads and acephalic sperms which can be seen in SDS treated mice under 40X magnification

4.2 Biochemical Study

4.2.1 Nitrite (ROS) Content (Quantitative)

The Nitrite content in control mice was **29.0644** µM. In case of positive control of β- carotene the Nitrite content was **30.9776** µM. The Nitrite content was found to be considerably increased in SDS fed mice being **70.3408** µM. In the mice fed with β- carotene and SDS the Nitrite content was **57.3624** µM. The statistical significances are denoted in the following table.

Table 8 Nitrite content in liver

Treatment series	Nitrite content (µM)
Control mice	29.064* ±0.121
β- carotene treated mice	30.978* ± 0.273
SDS treated mice	70.340* ± 0.174
β- carotene + SDS treated mice	57.362* ± 0.145

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.0001]

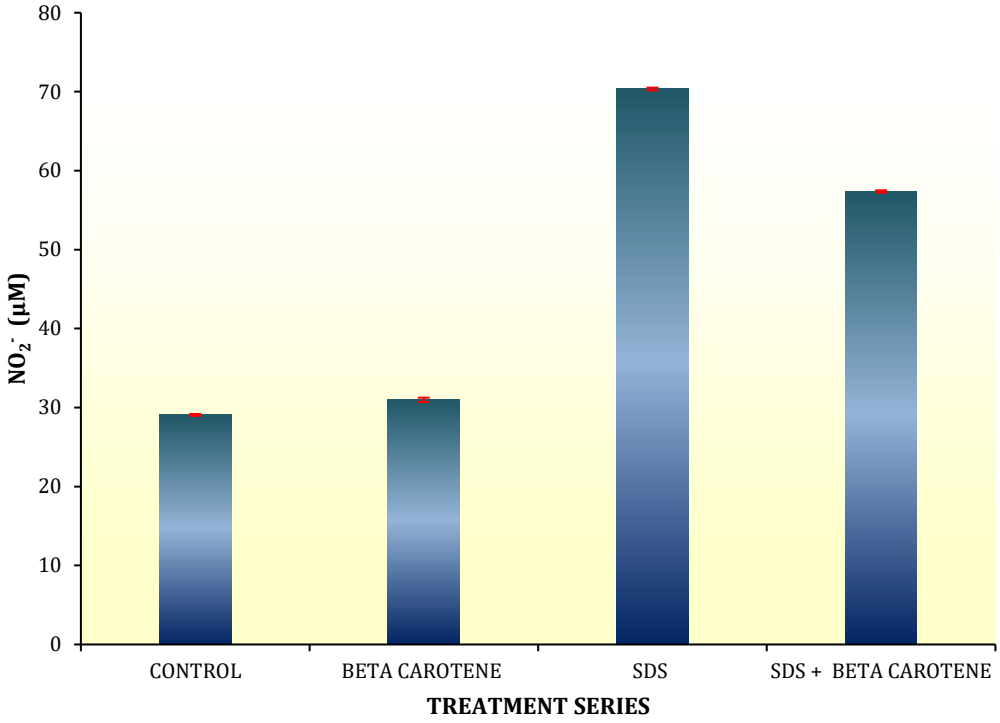


Fig. 10 Graphical representation of Nitrite content (µM) in liver

4.2.2 Nitrite Production (ROS content) in testis tissue

The Nitrite content in control mice was **21.4046** µM. In case of positive control of β- carotene the Nitrite content was **20.9782** µM. The Nitrite content was found to be considerably increased in SDS fed mice being **47.9902** µM. In the mice fed with β- carotene and SDS the Nitrite content was **37.5882** µM. The statistical significances are denoted in the following table.

Table 9 Nitrite content in testis

Treatment series	Nitrite content (µM)
Control mice	21.4046* ± 0.4443
β- carotene treated mice	20.9782*± 0.3239
SDS treated mice	47.9902*± 0.3188
β- carotene + SDS treated mice	37.5882 ± 0.2818

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.0001]

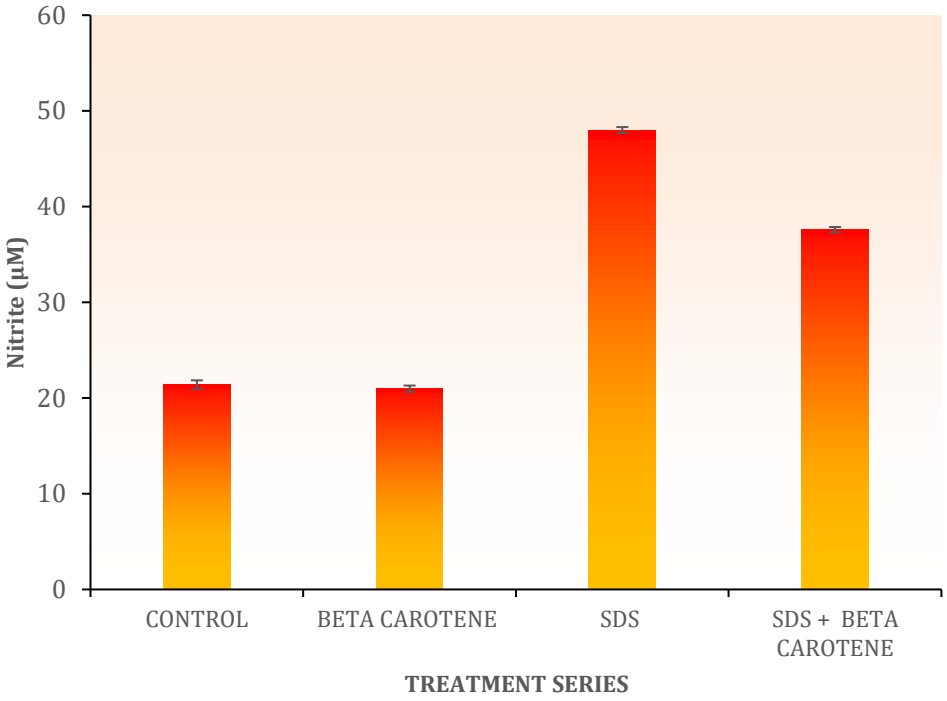


Fig. 11 Graphical representation of Nitrite content in testis (µM)

4.2.3 Catalase Enzyme Activity (Quantitative)

The catalase enzyme activity in control mice was **6.06 U/ml**. In case of positive control of β- carotene the catalase enzyme activity was **6.111 U/ml**. The catalase enzyme activity was found to be considerably reduced in SDS fed mice being **2.95 U/ml**. In the mice fed with β- carotene and SDS the catalase enzyme activity was **5.593 U/ml**. The statistical significances are denoted in the following table.

Table 10 Catalase Activity

Treatment series	Catalase Activity (U/ml)
Control mice	6.06 ± 0.056
β- carotene treated mice	6.111* ± 0.085
SDS treated mice	2.950* ± 0.058
β- carotene + SDS treated mice	5.593* ± 0.059

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.001 and P < 0.0001]

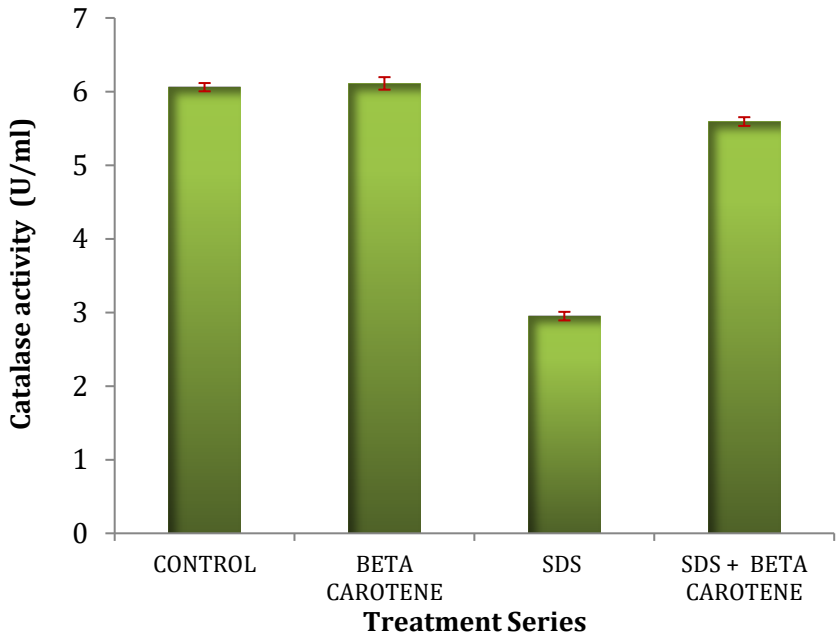


Fig. 12 Graphical representation of Catalase enzyme activity (U/ml)

4.2.4 Tissue protein Content (Quantitative)

The tissue protein level in control mice was **1.1624** mg/ml. In case of positive control of β- carotene the protein content was **1.2792** mg/ml. The protein level was found to be considerably reduced in SDS fed mice being **0.6912** mg/ml. In the mice fed with β- carotene and SDS the protein content was **0.9824** mg/ml. The statistical significances are denoted in the following table.

Table 11 Tissue protein level

Treatment series	Tissue Protein Level (mg/ml)
Control mice	1.1624* ±0.00858
β- carotene treated mice	1.2792* ± 0.00657
SDS treated mice	0.6912* ±0.01344
β- carotene + SDS treated mice	0.9824* ± 0.00521

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.0001]

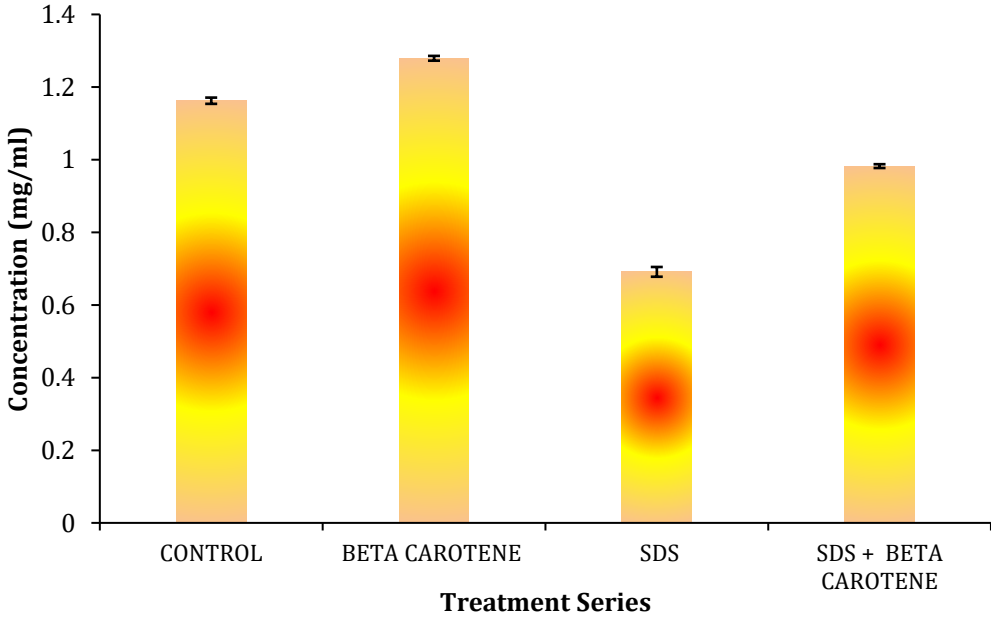


Fig. 13 Graphical representation of Tissue protein level (mg/ml)

4.3 Haematological Study

4.3.1 Total Erythrocyte Count (TEC)

In control mice, the total RBC count was found to be **5.4364×10^6** cells/mm^3, which is within the normal range. In β-carotene fed mice β- carotene treatment increases RBC count, i.e. **5.6748×10^6** cells/mm^3, but in SDS treated mice SDS treatment caused a decrease in erythrocyte count, i.e. **5.3626×10^6** cells/mm^3.The total RBC count in mice treated with both SDS and β-carotene was found to be **5.4358×10^6** cells/mm^3. The statistical significances are denoted in the following table.

Table 12 Number of RBC in control and treated groups

Treatment series	No. of RBCs / mm^3
Control mice	5.4364* ± 0.0176 × 10^6
β- carotene treated mice	5.6748* ± 0.0142 × 10^6
SDS treated mice	5.3626* ± 0.0059 × 10^6
β- carotene + SDS treated mice	5.4358* ± 0.0074 × 10^6

[Each value is expressed as the mean ± SE (n = 5 per group). Results were statistically analyzed with one-way ANOVA and Tukey HSD Test and compared with the control group when P < 0.05]

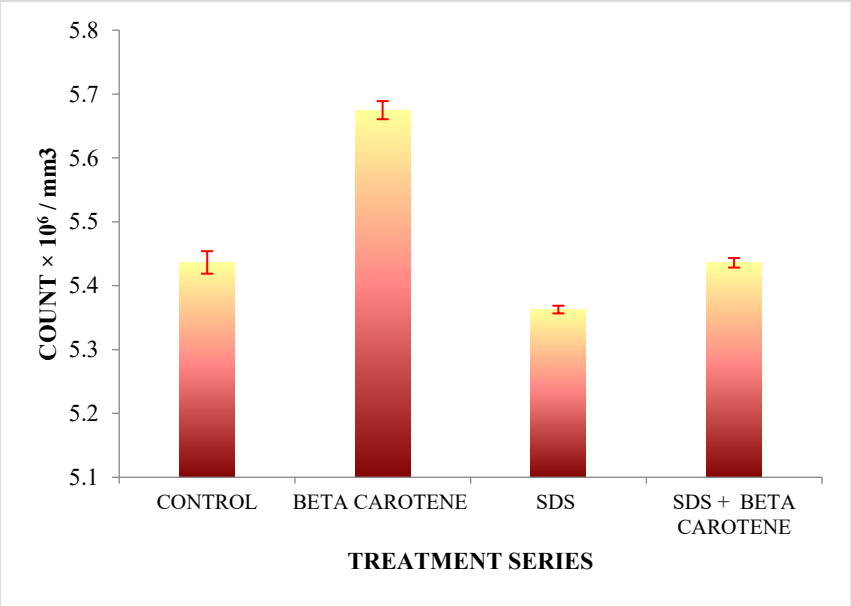


Fig. 14 Graphical representation of Total Erythrocyte Count (TEC)

4.3.2 Total Leukocyte Count (TLC)

In control mice, the total WBC count was found to be **6.2726 × 10³** cells/ mm³, which is within the normal range. In β-carotene fed mice leukocyte count increases, i.e., **6.4714 × 10³** cells/mm³, but in SDS treated mice, SDS treatment caused a decrease in leukocyte count, i.e., **4.1576 × 10³** cells/mm³, which was again restored in mice treated with both SDS and β-carotene. The total WBC count in mice treated with both SDS and β-carotene was found to be **5.1284 × 10³** cells/mm³. The statistical significances are denoted in the following table.

Table 13 Number of WBC in control and treated groups

Treatment series	No. of WBCs / mm³
Control mice	6.2726* ± 0.0038 × 10³
β- carotene treated mice	6.4714* ± 0.0020 × 10³
SDS treated mice	4.1576* ± 0.0086 × 10³
β- carotene + SDS treated mice	5.1284* ± 0.0133 × 10³

[Each value is expressed as the mean ± S.E. (n = 5 per group). Results were statistically analyzed with one way ANOVA and Tukey HSD Test and compared with the control group when P < 0.0001]

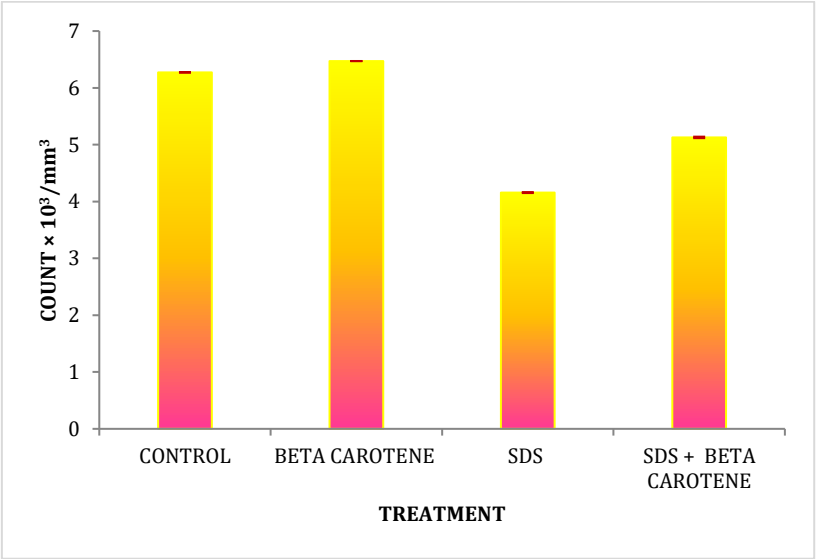


Fig. 15 Graphical representation of Total Leukocyte Count (TLC)

4.3.3 Differential Count of Leukocytes

In male Swiss albino mice, treatment with SDS induced significant shifts in the differential leukocyte count when compared to the control group. Neutrophils and eosinophils increased while lymphocytes decreased after SDS therapy, suggesting inflammation and mild immunological suppression. Mice administered with β-carotene

alone had reduced neutrophil as well as eosinophil levels and increased lymphocyte counts, indicating an anti-inflammatory and immune-stimulating impact. When SDS and β-carotene was given combined, both neutrophil and eosinophil number decreased in comparison to the SDS group, and the lymphocyte count restored to nearly normal.

Table 14 Differential Count of Control and treated groups

Treatment Series	Lymphocyte	Neutrophil	Monocyte	Eosinophil	Basophil
Control	161±0.71*	19.8±0.38*	8.2±0.2*	8.2±0.2*	2.8±0.2*
β-Carotene	168.8±0.8*	23±0.45*	3.6±0.25*	3.4±0.25*	1.2±0.2*
SDS	151.2±0.87	29.8±0.67	7.2±0.2*	9.6±0.4*	2.2±0.2*
SDS + β-Carotene	161.2±0.59*	22.2±0.38*	7.2±0.2	6.8±0.38*	2.6±0.25*

[Each value is expressed as the mean ± S.E. (n = 5 per group). Results were statistically analyzed with one way ANOVA and Tukey HSD Test and compared with the control group when P < 0.05]

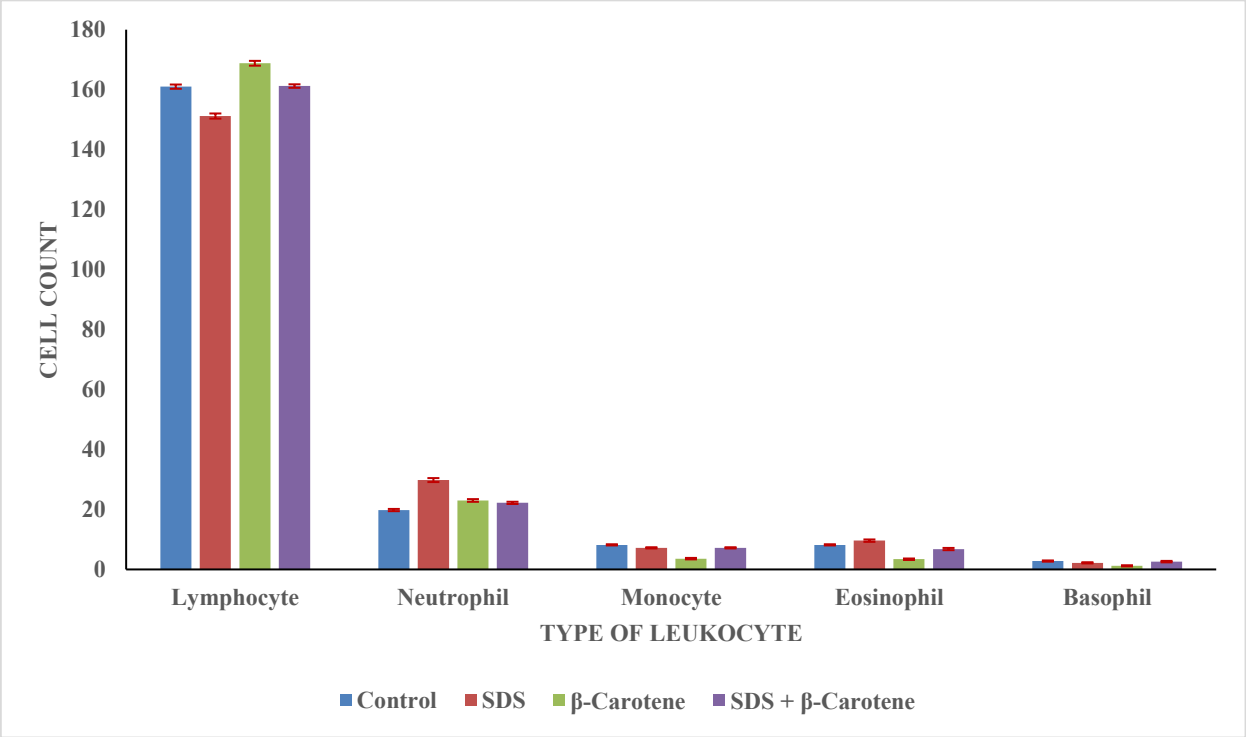


Fig. 16 Graphical representation of Differential Count

4.3.4 Haemoglobin Concentration

In Control mice, haemoglobin concentration was measured to be **12.88** g/dL, which is within the normal range. In β - carotene fed mice β- carotene caused an increase in haemoglobin concentration, i.e., **13.04** g/dL but in SDS treated mice, SDS treatment caused a decrease in haemoglobin concentration, i.e., **11.68** g/dL which was again restored in mice treated with both SDS and β-carotene. The haemoglobin concentration in mice treated with both SDS and β- carotene was found to be **12.16** g/dL. This result gives an indication that SDS acts as a haemoglobin suppressor agent, while β- carotene can be a potential haemoglobin inducer agent. The statistical significances are denoted in the following table.

Table 15 Concentration of haemoglobin in control and treated groups

Treatment series	Haemoglobin Concentration (g/dl)
Control mice	12.88* ± 0.1019
β- carotene treated mice	13.04 ± 0.1166
SDS treated mice	11.68* ± 0.08
β- carotene + SDS treated mice	12.16* ± 0.1166

[Each value is expressed as the mean ± S.E. (n = 5 per group). Results were statistically analyzed with one way ANOVA and Tukey HSD Test and compared with the control group when P < 0.05]

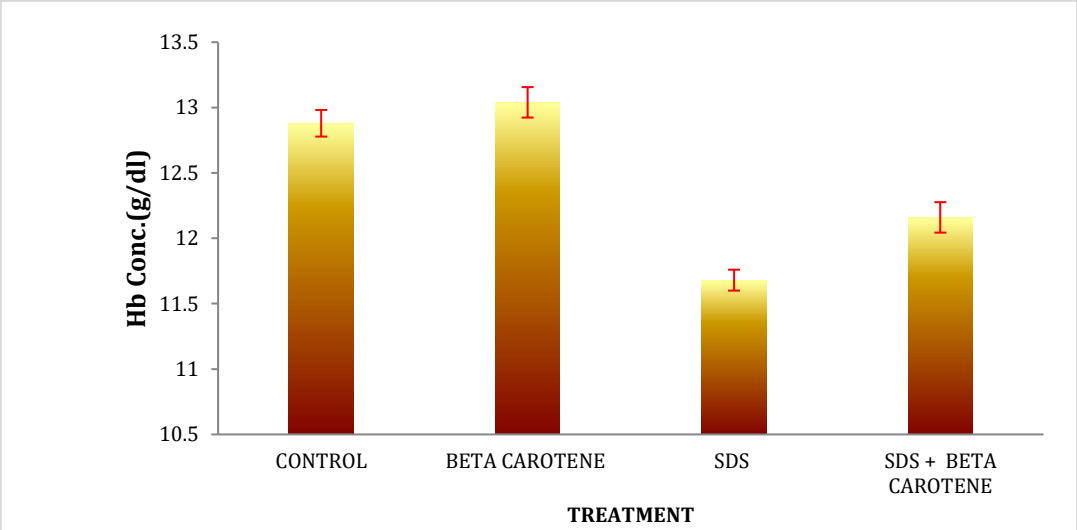


Fig. 17 Graphical representation hemoglobin concentration (g/dL)

4.4 Histological Study

4.4.1 Changes in Testis

The testicular sections of male mice, treated with SDS, reveals noteworthy changes in the seminiferous tubules, including structural disorganization, sloughing off the lumen and increased space between the seminiferous tubules. Several cells display hyperpycnotic nuclei, excessive vacuolization in the germinal layers. The treatment of these affected mice with β-carotene, showed to have a supposed enhancement of the antioxidant or the detoxification system, which consequently showed to have reduced the damage due to oxidative stress by SDS. Normal histoarchitecture has been found in β-carotene treated sections.

Table 16 Statistical representation of changes observed in Testis Tissue sections

	Lumen diameter (in µm)	Thickness of cell layer (in µm)	Diameter of seminiferous tubule (in µm)
Control	233.47 ± 2.85	104.18 ± 3.95	455.37 ± 12.43
Beta Carotene	231.09 ± 9.35	115.33 ± 7.61	453.59 ± 14.08
SDS	97.33 ± 3.79	80.33 ± 6.17	258.19 ± 7.46
SDS & Beta Carotene	114.53 ± 3.05	96.08 ± 6.01	308.66 ± 3.82

[Each value is expressed as the mean ± S.E. (n = 5 per group). Results were statistically analyzed with one way ANOVA and Tukey HSD Test and compared with the control group when P < 0.05]

Lumen diameter of testis excessively decreases with SDS treatment. In β-carotene treated section the lumen diameter remains as same as the control. In SDS and β-carotene treated sections show moderate decrease in lumen diameter. In SDS treated sections of testis the thickness of cell layer decreases. In beta carotene treated section the thickness of cell layer remains normal. In SDS and β-carotene treated section the decrease in the thickness of cell layer is moderate. Diameter of seminiferous tubules in SDS treated sections of testis shows excessive decrease. In beta carotene treated sections of testis the diameter of seminiferous tubules maintains normality. In SDS and β--carotene treated sections of testis the diameter of seminiferous tubule decreases moderately.

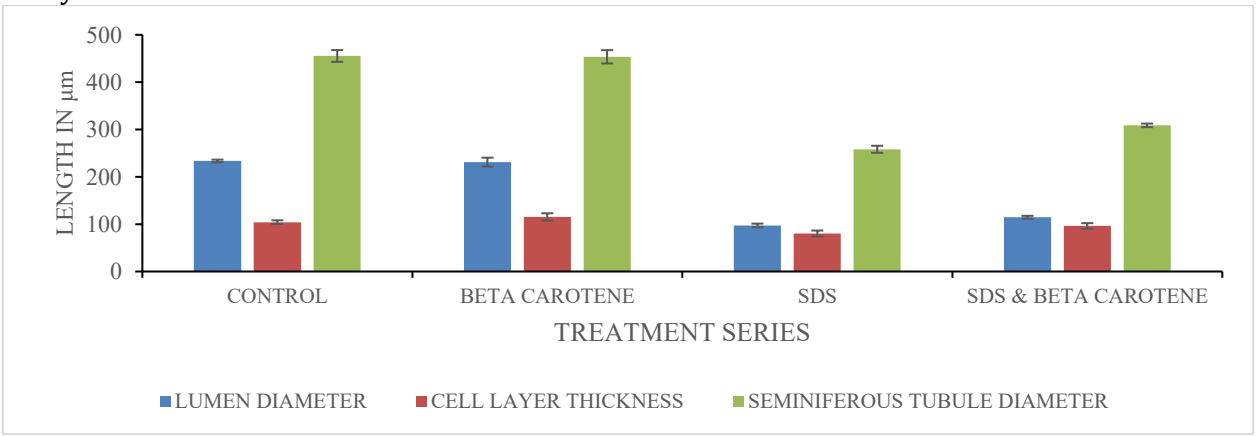


Fig. 18 Graphical representation of changes in testis observed statistically

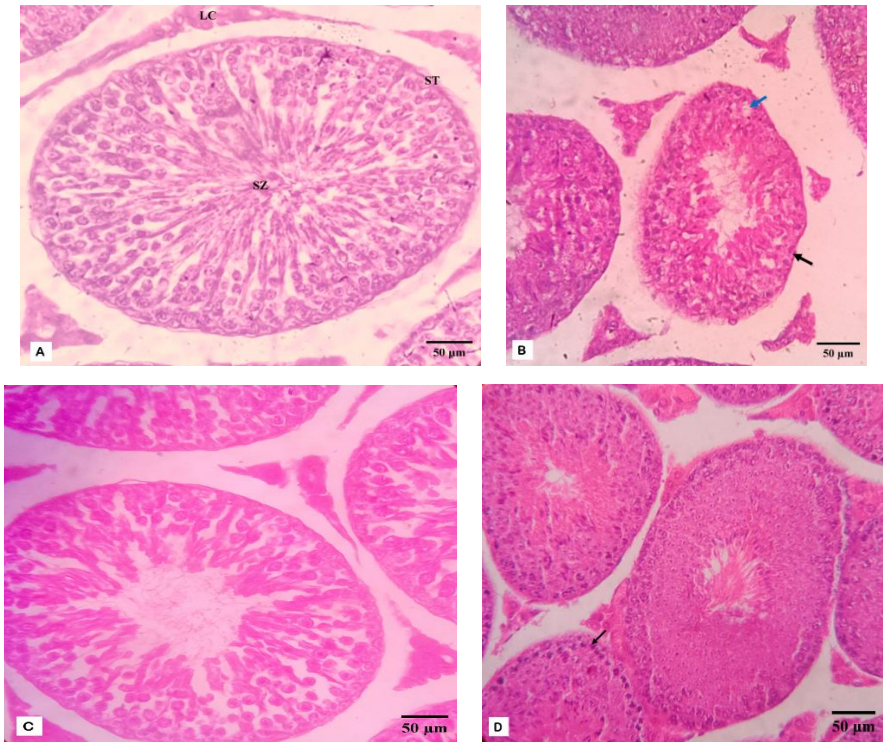
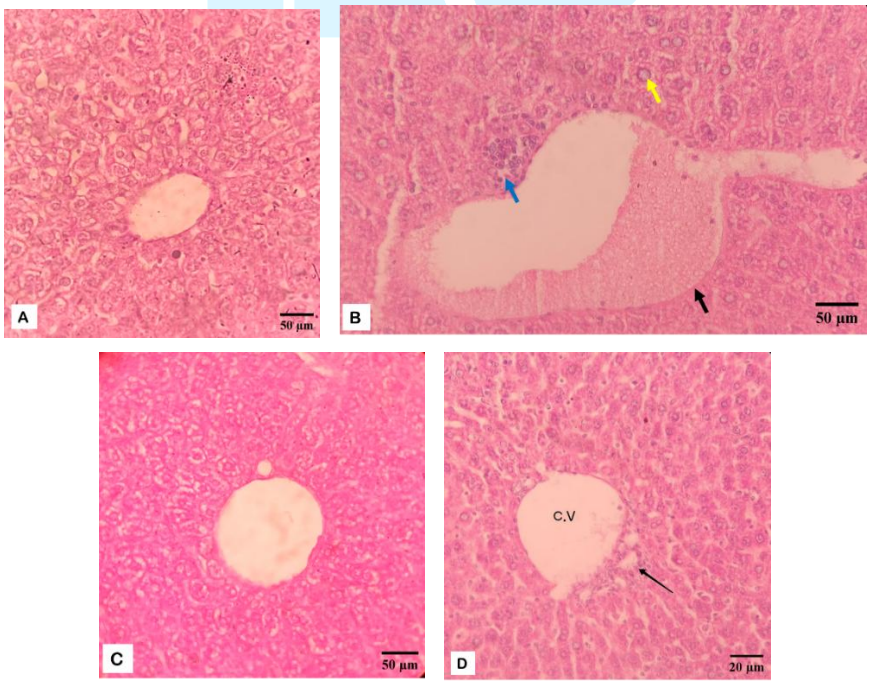


Fig. 19 Photomicrographs of histological analysis of testes; [A] The sectional view of testes from control Swiss albino mice showing normal seminiferous tubules (ST), interstitial cells of Leydig (LC) and spermatozoa (SZ), HE staining (40X); [B] Testes of mice treated with SDS, showing degeneration of seminiferous tubules (black arrow) and vacuolization (blue arrow), HE staining (40X); [C] Testes of mice treated with β-carotene showing normal histological structures, HE staining (40X); [D] Testes from mice treated with SDS and β -carotene showing hyperpycnotic nuclei (black arrow), HE staining (40X)

4.4.2 Changes in Liver

The liver sections of male mice, treated with SDS, reveals noteworthy changes in the central vein has been found like vascular congestion and dilation of the central vein. Additionally, vacuoles have been found in the hepatocytes and mononuclear cell infiltration has been seen around the bile duct. The treatment of these affected mice with β -carotene, showed to have a supposed enhancement of the antioxidant or the detoxification system, which consequently showed to have reduced the damage due to oxidative stress by SDS. Normal histoarchitechture has been found in β - carotene treated sections.



Fig, 20 Photomicrographs of histological analysis of liver; [A] The sectional view of liver from control Swiss albino mice showing normal hepatocytes, HE staining (40X); [B] Liver of mice treated with SDS, showing vascular congestion (black arrow) and dilation of central vein (CV), vacuoles in hepatocytes (yellow arrow) and mononuclear cell infiltration (blue arrow), HE staining (40X); [C] Liver of mice treated with β -carotene showing normal hepatocytes, HE staining (40X); [D] Liver mice treated with SDS and β -carotene showing bile duct (black arrow), HE staining (40X)

Hepatocyte diameter increased markedly in SDS-treated liver (29.52 µm) compared to control (21.15 µm), while β-carotene treatment maintained near-control values (21.98 µm). In SDS and β-carotene co-treatment, hepatocyte diameter showed a moderate increase (25.78 µm).

Central vein diameter was drastically enlarged in SDS-treated sections (293.44 µm) compared to control (145.85 µm). β-carotene maintained near-normal diameter (138.83 µm), whereas co-treatment showed moderate enlargement (240.65 µm).

Table 17 Statistical representation of changes observed in Liver Tissue sections

Treatment Series	Hepatocyte Diameter In Mouse Liver (in µm)	Central Vein Diameter In Mouse Liver (in µm)
Control	21.1582 ± 1.01*	145.8574 ± 2.23*
β-carotene	21.9822 ± 0.4*	138.8362 ± 2.48*
SDS	29.521 ± 0.58	293.4478 ± 4.55*
SDS & β-carotene	25.7802 ± 0.92*	240.6512 ± 2.94*

[Each value is expressed as the mean ± S.E. (n = 5 per group). Results were statistically analyzed with one way ANOVA and Tukey HSD Test and compared with the control group when P < 0.05]

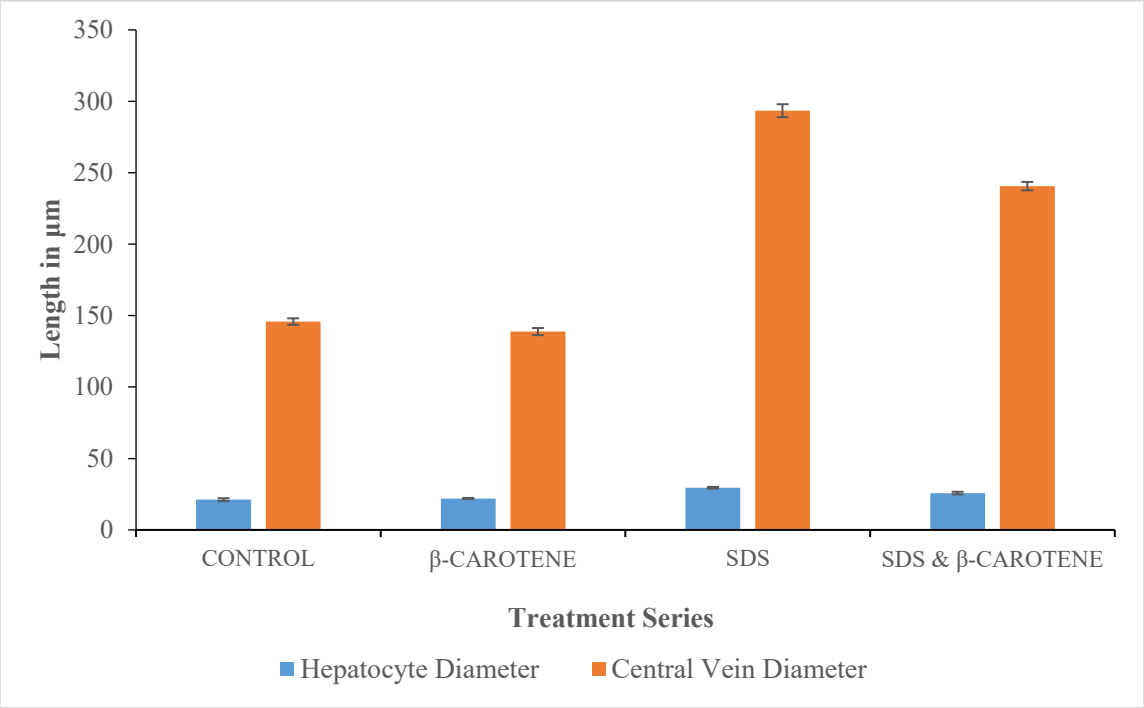


Fig. 21 Graphical representation of changes in liver observed statistically

4.4.3 Changes in Pancreas

The pancreas sections of male mice, treated with SDS, under the microscope reveals noteworthy changes in the lobules, including degeneration of lobular architecture. Also, fluid accumulation can be seen in between the lobules and vacuolization of acinar cells. Additionally, the ducts have been dilated. The treatment of these affected mice with β- carotene, showed to have a supposed enhancement of the antioxidant or the detoxification system, which consequently showed to have reduced the damage due to oxidative stress by SDS. Normal histoarchitecture has been found in β- carotene treated sections.

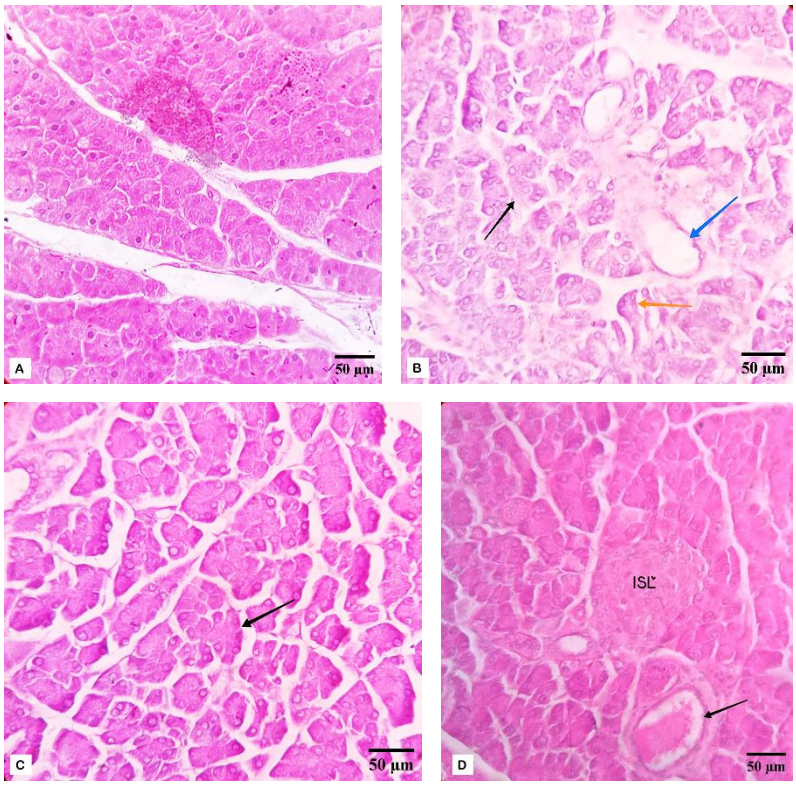


Fig. 22 Photomicrographs of histological analysis of pancreas; [A] The sectional view of pancreas from control Swiss albino mice showing normal acinar cells (black arrow), HE staining (40X); [B] Pancreas of mice treated with SDS, showing degeneration of lobular structure (orange arrow), dilated ducts (blue arrow) and fluid accumulation (black arrow), HE staining (40X); [C] Pancreas of mice treated with β- carotene showing normal histological structures (black arrow), HE staining (40X); [D] Pancreas of mice treated with SDS and β- carotene showing islets of Langerhans (ISL) and dilated duct (black arrow), HE staining (40X)

5. Discussion

The present study was designed to investigate the **stress related** effects of Sodium Dodecyl Sulfate (SDS) on various biological systems of male Swiss albino mice and to explore the potential of β-carotene in counteracting these effects. A range of parameters was assessed to understand the impact on cytological, physiological and biochemical parameters. The observations obtained from histopathological, hematological, cytological, and biochemical investigations clearly indicate that SDS induces significant stress, while β-carotene shows promising protective effects.

Histopathological observations revealed that SDS caused visible structural damage in the liver, testis, and pancreas. The liver showed signs of vascular congestion, vacuolated hepatocytes, and inflammatory cell infiltration. The testes exhibited degeneration of seminiferous tubules and vacuolization of germinal layers. The pancreas showed disrupted lobular structure, vacuolization of acinar cells, and fluid accumulation. These structural changes suggest **oxidative and inflammatory damage** caused by SDS. However, in animals co-treated with β-carotene, the histological structure was significantly improved, showing more organized tissues and fewer pathological signs, supporting the protective role of β-carotene against cellular damage.

SDS exposure also affected blood parameters. A decrease in red blood cell counts and haemoglobin concentration indicated the development of anaemia or disruption in erythropoiesis. Also, we found a decrease in white blood cell count which might indicate a threat to the immune system. The group that received β-carotene along with SDS showed a marked improvement in these values, indicating the compound's supportive effect on hematopoietic and immune function. Decreased lymphocytes, increased neutrophils and eosinophils indicate the probable immunological imbalance in SDS treated mice. Combined treatment with β-carotene modified as well as reversed these alterations in some cases. Leukocyte levels returned to normalcy after the combination treatment, suggesting that β-carotene successfully mitigates the immunological stress caused by SDS.

Cytological analysis revealed that SDS had a strong negative impact on male reproductive cells. The sperm count dropped significantly, motility was reduced, and a sharp rise in the percentage of sperm head abnormalities was noted. These outcomes point toward reproductive toxicity, likely driven by oxidative stress and disruption of cytoskeletal structures. However, co-administration of β-carotene greatly improved sperm quality, increasing count and motility while reducing abnormal forms. This

supports the idea that β-carotene helps maintain the integrity of reproductive cells.

Further, SDS caused a considerable drop in the number of viable bone marrow cells and led to increased formation of micronuclei and chromosomal abnormalities. These findings confirm its genotoxic effects and suggest potential long-term consequences if exposure continues. β-carotene, however, improved cell viability and reduced chromosomal aberrations under oxidative conditions to a certain level.

Biochemical tests confirmed that SDS significantly increased the levels of reactive oxygen species, as seen from elevated nitrite content in the liver and testis. At the same time, catalase activity, an important antioxidant defence enzyme was reduced. Protein levels in liver tissue also dropped, likely due to protein degradation or impaired synthesis. These results indicate that SDS induces oxidative stress, damages proteins, and disrupts enzyme function. Treatment with β-carotene reversed many of these effects by lowering nitrite levels, restoring catalase activity, and increasing protein content, further validating its antioxidative role.

6. Conclusion

From the results of this study, it is evident that SDS causes wide ranging stress-associated toxic effects on the health of male Swiss albino mice, affecting growth, organ function, blood profile, sperm quality, genetic integrity and oxidative balance. These adverse effects appear to be closely linked with oxidative stress and cellular damage. β-carotene, through its antioxidant properties, was able to reduce or prevent many of these effects. It helped maintain body and organ weights, improved tissue structure, normalized haematological and cytological parameters, and reduced oxidative and chromosomal damage. These findings suggest that β-carotene may serve as a useful dietary supplement in protecting the harmful effects of chemical exposures like SDS. However, further research with long term exposure is needed to determine their most effective dosage, explore its long-term safety, and understand the molecular pathways through which it offers protection.

Disclaimer (Artificial Intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Ethical Approval

We have taken necessary ethical permission from The Animal Welfare Committee, Maulana Azad College for using experimental albino rats during our study. Content of the manuscript is original and has not been accepted or published somewhere, either in whole or in part. The manuscript is not submitted elsewhere for evaluation for publication.

Acknowledgement and Funding

We acknowledge our indebtedness, deep sense of gratitude and sincere regards to the Head, Department of Zoology, Maulana Azad College for financial support of the work.

Competing Interests

Authors have declared that no competing interests exist.

References

1. Agner, T. (1991). Susceptibility of atopic dermatitis patients to irritant dermatitis caused by sodium lauryl sulphate. Acta Dermato-Venereologica, 71(4), 296–300. https://doi.org/10.2340/0001555571296300
2. Arai, S., Caamaño, S., Strauss, S. H., & Orton, C. (2009). Does sodium dodecyl sulfate wash out of detergent-treated bovine pericardium at csssssssssytotoxic concentrations? The Journal of Heart Valve Disease, 18(1), 101–105.
3. Ashok, V. D., & De, S. K. (2011). Structure of the Sodium Dodecyl Sulfate Surfactant on a Solid Surface in Different NaCl Solutions. Langmuir, 27(43), 12422–12428. https://doi.org/10.1021/la900714a
4. Bendich, A., & Shapiro, S. S. (1986). Effect of β-carotene and canthaxanthin on the immune responses of the rat. Journal of Nutrition, 116(10), 2254–2262.
5. Bidevkina, M. V., Golubeva, M. I., Limantsev, A. v., Razumnaya, I. N., Potapova, T. N., &Fedorova, E. A. (2020). Assessment of the toxicity and hazard of sodium lauryl sulfate at different exposure routes. Toxicological Review, 4. https://doi.org/10.36946/0869-7922-2020-4-56-59
6. Bossy-Wetzel, E., & Lipton, S. A. (2003). Nitric oxide signaling regulates mitochondrial number and function. Cell Death and Differentiation, 10(7). https://doi.org/10.1038/sj.cdd.4401244
7. Brevard, P. B. (1994). β-Carotene increases monocyte numbers in peripheral rat blood. International Journal for Vitamin and Nutrition Research, 64(2), 104–108.
8. Chelikani, P., Fita, I., & Loewen, P. C. (2004). Diversity of structures and properties among catalases. In Cellular and Molecular Life Sciences (Vol. 61, Issue 2). https://doi.org/10.1007/s00018-003-3206-5
9. Chisté, R. C., Freitas, M., Mercadante, A. Z., & Fernandes, E. (2014). Carotenoids inhibit lipid peroxidation and hemoglobin oxidation, but not the depletion of glutathione induced by reactive oxygen species in human erythrocytes. Life Sciences, 99(1–2), 52–60.

10. Dayer, M. R., Moosavi-Movahedi, A. A., Norouzi, P., Ghourchian, F., Hedayat-Olah, H., & Safarian, S. (2002, July 31). Inhibition of human hemoglobin autoxidation by sodium n-dodecyl sulphate. BMB Reports, 35(4), 364–370.
11. Dhruv, D. (2023). The Study of Sodium Lauryl Sulfate (SLS) Toxicity. Journal of Clinical Toxicology, 13(4).
12. García-Casal, M. N., Layrisse, M., Solano, L., Barón, M. A., Arguello, F., Llovera, D., Ramírez, J., Leets, I., & Tropper, E. (1998). Vitamin A and β-carotene can improve nonheme iron absorption from rice, wheat and corn by humans. The Journal of Nutrition, 128(3), 646–650.
13. Ghyasvand, T., Goodarzi, M. T., Amiri, I., Karimi, J., &Ghorbani, M. (2015). Serum levels of lycopene, beta-carotene, and retinol and their correlation with sperm DNA damage in normospermic and infertile men. International journal of reproductive biomedicine, 13(12), 787–792.
14. Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Analytical biochemistry, 126(1), 131–138. https://doi.org/10.1016/0003-2697(82)90118-x
15. Gumpricht, E., Dahl, R., Devereaux, M. W., &Sokol, R. J. (2004). Beta-carotene prevents bile acid-induced cytotoxicity in the rat hepatocyte: Evidence for an antioxidant and anti-apoptotic role of beta-carotene in vitro. Pediatric research, 55(5), 814–821. https://doi.org/10.1203/01.PDR.0000117845.23762.6B
16. Hadwan, M. H. (2018). Simple spectrophotometric assay for measuring catalase activity in biological tissues. BMC Biochemistry, 19(1). https://doi.org/10.1186/s12858-018-0097-5
17. Halestrap, A. P. (2009). Mitochondria and reperfusion injury of the heart-A holey death but not beyond salvation. In Journal of Bioenergetics and Biomembranes (Vol. 41, Issue 2). https://doi.org/10.1007/s10863-009-9206-x
18. Hiner, A. N., Hernández-Ruiz, J., Rodríguez-López, J. N., García-Cánovas, F., Brisset, N. C., Smith, A. T., Arnao, M. B., & Acosta, M. (2002). Reactions of the class II peroxidases, lignin peroxidase and Arthromyces ramosus peroxidase, with hydrogen peroxide. Catalase-like activity, compound III formation, and enzyme inactivation. *The Journal of biological chemistry*, *277*(30), 26879–26885. https://doi.org/10.1074/jbc.M200002200
19. Irizarry Rovira, A. R., Hilbish, K. G., Schroeder, M., Boorman, G. A., Credille, K. M., Ballard, D., Hanson, J. C., &Niedenthal, A. (2021). Effects of 0.5% and 2.0% Sodium Lauryl Sulfate in Male CD-1 Mice From a 3-Month Oral Gavage Toxicity Study. Toxicologic pathology, 49(5), 1100–1108. https://doi.org/10.1177/01926233211004873
20. JossonAkkara, P., & Sabina, E. P. (2020). A biochemical approach to the anti-inflammatory, antioxidant and antiapoptotic potential of beta-carotene as a protective agent against bromobenzene-induced hepatotoxicity in female Wistar albino rats. Journal of applied biomedicine, 18(2-3), 87–95. https://doi.org/10.32725/jab.2020.011
21. Kheir-Eldin, A. A., Motawi, T. K., Gad, M. Z., & Abd-Elgawad, H. M. (2001). Protective effect of vitamin E, β-carotene and N-acetylcysteine from the brain oxidative stress induced in rats by lipopolysaccharide. International Journal of Biochemistry and Cell Biology, 33(5). https://doi.org/10.1016/S1357-2725(01)00032-2
22. Kumar, S., Kirha, T. J., & Thonger, T. (2014). Toxicological effects of sodium dodecyl sulfate. Journal of Chemical and Pharmaceutical Research, 6(5).
23. Lawlor, S. M., &O'brien, N. M. (1995). Modulation of oxidative stress by β-carotene in chicken embryo fibroblasts. British Journal of Nutrition, 73(6). https://doi.org/10.1079/bjn19950089
24. Lin, W. T., Huang, C. C., Lin, T. J., Lin, M. T., & Chen, W. K. (2016). Enhanced protective effects of combined treatment with β-carotene and curcumin against hyperthermic spermatogenic disorders in mice. BioMed Research International, 2016, 2572073.
25. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. The Journal of biological chemistry, 193(1), 265–275.
26. Michal, J. J., Heirman, L. R., Wong, T. S., Chew, B. P., Frigg, M., & Volker, L. (1994, May). Modulatory effects of dietary β-carotene on blood and mammary leukocyte function in periparturient dairy cows. Journal of Dairy Science, 77(5), 1408–1421.
27. Moghimi, Y., Banaei, A., Majdaeen, M., Zamani, H., & Abedi-Firouzjah, R. (2021). Radiation protection and cytotoxicity effects of different concentrations of cerium oxide nanoparticles in aqueous solution combined with sodium dodecyl sulphate in Vero cells irradiated with 18 MV beams. International Journal of Radiation Research, 19(4), 913-920.
28. Muzandu, K., Ishizuka, M., Sakamoto, K. Q., Shaban, Z., el Bohi, K., Kazusaka, A., & Fujita, S. (2006). Effect of lycopene and β-carotene on peroxynitrite-mediated cellular modifications. Toxicology and Applied Pharmacology, 215(3). https://doi.org/10.1016/j.taap.2006.03.006
29. Nakagawa, K., Fujimoto, K., & Miyazawa, T. (1996). β-Carotene as a high-potency antioxidant to prevent the formation of phospholipid hydroperoxides in red blood cells of mice. Biochimica et Biophysica Acta, 1299(1), 110–116.
30. Niu, X., Zheng, S., Liu, H., & Li, S. (2018). Protective effects of taurine against inflammation, apoptosis, and oxidative stress in brain injury. Molecular medicine reports, 18(5), 4516–4522. https://doi.org/10.3892/mmr.2018.9465
31. Poli, A. L., Moreira, L. M., Tabak, M., & Imasato, H. (2006). SDS (sodium dodecyl sulfate) effect on the autoxidation of the Glossoscolex paulistus giant extracellular hemoglobin: Kinetic studies at pH 7.0 and 9.0. Colloids and Surfaces B: Biointerfaces, 52(1), 96–104.
32. Salehi, N., Moosavi-Movahedi, A. A., Fotouhi, L., Yousefinejad, S., Shourian, M., Hosseinzadeh, R., Sheibani, N., & Habibi-Rezaei, M. (2014). Heme degradation upon production of endogenous hydrogen peroxide via interaction of hemoglobin with sodium dodecyl sulfate. Journal of Photochemistry and Photobiology B: Biology, 133, 11–17.

33. Salvadori, D. M., Ribeiro, L. R., Oliveira, M. D., Pereira, C. A., &Beçak, W. (1992). The protective effect of beta-carotene on genotoxicity induced by cyclophosphamide. Mutation research, 265(2), 237–244. https://doi.org/10.1016/0027-5107(92)90052-4
34. Sandoval, C., Mella, L., Godoy, K., Hidalgo, J., & Vera, A. (2022). β-Carotene increases activity of cytochrome P450 2E1 during ethanol consumption and improves hepatic oxidative balance. Antioxidants, 11(5), 1033.
35. Sandoval, C., Vera, A., Birditt, K., Godoy, K., Carmine, F., Caamaño, J., & Farías, J. (2024). β-Carotene Supplementation Improves Pancreas Function during Moderate Ethanol Consumption: Initial Characterization from a Morphological Overview. International journal of molecular sciences, 25(2), 1219. https://doi.org/10.3390/ijms25021219
36. Sarada, S. K., Dipti, P., Anju, B., Pauline, T., Kain, A. K., Sairam, M., Sharma, S. K., Ilavazhagan, G., Kumar, D., & Selvamurthy, W. (2002). Antioxidant effect of beta-carotene on hypoxia induced oxidative stress in male albino rats. *Journal of ethnopharmacology*, *79*(2), 149–153. https://doi.org/10.1016/s0378-8741(01)00360-9
37. Schriner, S. E., Linford, N. J., Martin, G. M., Treuting, P., Ogburn, C. E., Emond, M., Coskun, P. E., Ladiges, W., Wolf, N., Van Remmen, H., Wallace, D. C., & Rabinovitch, P. S. (2005). Extension of murine life span by overexpression of catalase targeted to mitochondria. Science (New York, N.Y.), 308(5730), 1909–1911. https://doi.org/10.1126/science.1106653
38. Sena, L. A., & Chandel, N. S. (2012). Physiological roles of mitochondrial reactive oxygen species. In Molecular Cell (Vol. 48, Issue 2). https://doi.org/10.1016/j.molcel.2012.09.025
39. Siems, W. G., Sommerburg, O., Schild, L., Augustin, W., Langhans, C. D., &Wiswedel, I. (2002). Beta-carotene cleavage products induce oxidative stress in vitro by impairing mitochondrial respiration. Free Radical Biology and Medicine, 33(8), 1023–1032. https://doi.org/10.1016/s0891-5849(02)00902-6
40. Sokol, R. Z., Shulman, P., & Paulson, R. J. (2000). Comparison of two methods for the measurement of sperm concentration. Fertility and Sterility, 73(3). https://doi.org/10.1016/S0015-0282(99)00590-7
41. Stadtman, E. R., & Levine, R. L. (2000). Protein oxidation. *Annals of the New York Academy of Sciences*, *899*, 191–208. https://doi.org/10.1111/j.1749-6632.2000.tb06187.x
42. Starkov, A. A. (2008). The role of mitochondria in reactive oxygen species metabolism and signaling. Annals of the New York Academy of Sciences, 1147, 37–52.https://doi.org/10.1196/annals.1427.015
43. Su, L., Fang, W., Zhao, X., Zhu, L., Gao, L., & Chen, G. (2022). Disruption of mitochondrial redox homeostasis as a mechanism of antimony-induced reactive oxygen species and cytotoxicity. Ecotoxicology and environmental safety, 237, 113519. https://doi.org/10.1016/j.ecoenv.2022.113519
44. Svegliati-Baroni, G., Saccomanno, S., van Goor, H., Jansen, P., Benedetti, A., &Moshage, H. (2001). Involvement of reactive oxygen species and nitric oxide radicals in activation and proliferation of rat hepatic stellate cells. Liver, 21(1), 1–12. https://doi.org/10.1034/j.1600-0676.2001.210101.x
45. Tayfur, S., Al-Bayti, A., & Al-Bayati, A. (2020). Histopathological effect of powder detergent on the livers of laboratory mice (*Mus musculus*). EAI Endorsed Transactions on Pervasive Health and Technology, 6(23), e1–e6.
46. Tennant, J. R. (1964). Evaluation of the trypan blue technique for determination of cell viability. Transplantation, 2(6), 685-694.
47. Ugwoke, A. N., Anyaeze, C. M., Eze, E. I., Ugwoke, I. O., & Ugwu, O. P. (2020). Toxicological effects of liquid detergent on reproductive organs of male albino rats. Journal of Men's Health, 5(1), 15–20.
48. Umegaki, K., Takeuchi, N., Ikegami, S., & Ichikawa, T. (1994). Effect of β-carotene on spontaneous and x-ray-induced chromosomal damage in bone marrow cells of mice.
49. Wang, J., Wang, J., Xu, C., Liu, R., & Chen, Y. (2016). Molecular mechanism of catalase activity change under sodium dodecyl sulfate-induced oxidative stress in mouse primary hepatocytes. Journal of Hazardous Materials, 307, 173–183.
50. Wang, X., Wang, W., Pan, Y., Zhang, C., & Gao, J. (2020). Sodium taurocholate-induced severe acute pancreatitis in C57BL/6 mice. Journal of Visualized Experiments, (162), e61547.
51. Wyrobek, A. J., Watchmaker, G., & Gordon, L. (1984). An evaluation of sperm tests as indicators of germ-cell damage in men exposed to chemical or physical agents. Progress in clinical and biological research, 160, 385–405.
52. Yang, S. C., Huang, C. C., Chu, J. S., & Chen, J. R. (2004). Effects of β-carotene on cell viability and antioxidant status of hepatocytes from chronically ethanol-fed rats. British Journal of Nutrition, 92(2), 209-215.
53. Yang, W., & Acosta, D. (1995). A digitized fluorescence imaging study of intracellular Ca2+, pH, and mitochondrial function in primary cultures of rabbit corneal epithelial cells exposed to sodium dodecyl sulfate. In vitro cellular & developmental biology. Animal, 31(7), 499–507. https://doi.org/10.1007/BF02634027