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HAEMATOLOGICALAMELIORATIONBY*ZIZYPHUSMAURITIANA*INSILICAINDUCEDTOXICITYINWISTAR ALBINO RATS

Mr. Priyadarshini Karre, Ms. Yerravalli Priyanka, Mrs. Mala Sridevi
Assistant professor[1,2,3]
Department of Pharmacology, ,
Global College of Pharmacy, Hyderabad. Chilkur (V), Moinabad (M), Telangana- 501504

ABSTRACT: Aqueous extracts of Zizyphusmauritiana (ZM) roots, stems, and leaves were tested for their biochemical effects on silica-induced toxicity in rats, specifically looking for changes in inflammatory markers in the liver and kidney. For this study, wistar albino rats were given silica intraperitoneally and Zizyphusmauritiana extracts orally for 21 days to see if the extracts warded off silica-induced toxicity. Biochemical examination of blood samples was performed at the conclusion of the experiment. In comparison to the normal control group, animals given silica and Zizyphusmauritiana root, stem, and leaf extracts at the same time showed a marked reduction in serum levels of inflammatory cytokines TNF-α and IL-6, as well as ALT, AST, ALP, urea, uric acid, creatinine, and LDH. The animals given zizyphusmauritiana extracts showed a reduction in body weight, but the ones given silica showed no such effect. Ultimately, the results of this research showed that Zizyphusmauritiana bark extracts from roots, stems, and leaves might protect mice against the harmful effects of silica compared to animals given silica alone.

INTRODUCTION:

Exposure to silica on the job may induce silicosis, a kind of occupational lung disease that can cause both short-term and long-term respiratory problems. 1. An estimated 10 million Indian laborers get silicosis annually, according to reports 2. Because of its pervasiveness in both the workplace and everyday life, silica is considered one of the earth's most ubiquitous minerals. Workers in the mining, thermal power station, ceramic, cement, and rock drilling industries are at increased risk of health problems and death due to silica exposure. Inhalation, ingesting, dermal penetration, and injection are four routes of entry for silica into the human body. There have been significant increases in the usage of silica due to recent technical advancements, which have found widespread use in biomedical, drug delivery system 5, sensor, and commercial industries. 6. In most cases, exposure to silica has been linked to a wide range of health problems, including cancer, heart disease, lung illness, liver disease, kidney disease, and a host of others.

Exposure to silica, according to the available data, causes cirrhosis of the liver, characterized by particle buildup and metabolic abnormalities. The liver is the primary organ that xenobiotic substances biotransform. 7. Because silica builds up and causes oxidative stress, it also disrupts normal renal function, which affects kidney function 8 and increases free radical formation. An increase in the levels of liver and kidney marker enzymes in the blood indicates that the membrane integrity is disrupted by the free radicals that are so produced. Silica induced toxicity in humans does not have a viable therapy accessible at this time. The current course of therapy is mostly supportive, including the use of anti-inflammatory drugs to alleviate

discomfort. Despite promising results in curing the condition, this medication is often accompanied by unwanted side effects. Tribal people and low-income workers make up the bulk of the workforce in mining and related sectors. People in this economically disadvantaged class often succumb to illnesses because they do not have access to timely and proper treatment. The Ayurvedic method of treating illness has a long history of use in India. There is less of a chance of decreasing side effects and the financial burden of therapy when naturally occurring plant components are used. That is why we opted for the herbal approach, using the medicinal plant 'Zizyphusmauritiana' (ZM), to counteract the silica-induced toxicity.
This plant has a broad range of uses, particularly in areas prone to drought, and has been extensively studied for its potential therapeutic benefits. Fortunately, it has not been associated with any negative side effects. Alkaloids, ascorbic acid, and other phytochemicals abound in Zizyphusmauritiana. Zizyphusmauritiana produces analgesic effects in rats when diluted with water from its root barks 11, and anti-diarrheal effects when diluted with methanol from its stem barks 12. The anti-inflammatory and anti-allergenic effects of compounds extracted from the root bark have been shown. 13. We designed this study to evaluate the therapeutic effect of aqueous extracts of bark, roots, stem, and leaves of Zizyphusmauritiana. We used wistar albino rats as an animal model to see if it prevented silica-induced toxicity.

MATERIALSAND METHODS:

Chemicals: Silicon dioxide (SiO_2), ethanol, diethylether,chloroform,sodiumcarbonate,sodium bicarbonate,ethylenediamine-tetraaceticacid(EDTA),disodiumphenylphosphate,phenol,potassiumferricyanide,4-aminoantipyrine,reducednicotinamideadeninedinucleotidephosphate(NADPH),andsodiumpyruvate werepurchased from Sigma chem. Co. USA. All otherchemicalswereofanalyticalgrade.

Animals: Healthy maleWistaralbinorats(200-250g)wereobtainedfromNationalCentreforLaboratory Animal Sciences (NCLAS), Hyderabad,India.Animalsweremaintainedatstandard

conditions (temperature 25 ± 2 °C) with 12 h light /12 h dark cycle and fed *ad libitum* with standardpellet diet (Hindusthan Lever) and purified water,with free access to food and water. All the normsprescribedbyCOPCEA,GovernmentofIndia,were critically followed (vide the permission letterfrom Institutional Animal Ethics Committee, dated:12/3/2015).

Collection of Plant Material: Barks of roots andstemandmaturehealthyleavesof*Ziziphusmauritiana*(ZM) tree were collected from a singletreegrowinginforestofGadchirolidistrictof Maharashtra state (India) in the month of October2014.TheplantwasauthenticatedatUniversityDepartmentofBotany,RashtrasantTukadoji Maharaj Nagpur University, Nagpur and voucherspecimen no. 9138 was deposited in the herbarium.The plant was identified to be *Zizyphusmauritiana*Lam.(FamilyRhamnaceae).

PreparationofExtracts: Driedbarkswerecrushed in grinder and strained through the strainerto remove any hard part of the bark escaped duringgrinding.Leaveswereairdriedundershadeincontrolledconditionandcrushedtogetpowder.The powders were stored in air tight brown glasscontainerstoprotectfrommoistureanddirectsunlight. Ten percent aqueous extract was preparedbyboilingthepowderinwaterfor30minanddpassed through muslin cloth. The aqueous extractswerefilteredandsubjectedtorotaryvacuumevaporator(Superfit DB3135S).

Complete evaporation of water from extract wasachievedbydryingtheextractsatroomtemperatureundercontrolledconditionsbyspreadingincleanglasspetriplates.Thedriedscrapings were stored in sterilised airtight brownglassbottlesuntiluse.Atthetimeofusethescrapingswerecarefullyweighedonelectronicbalanceandsolubilised0.1Msodiumphosphatebuffered saline (pH 7.0), vortexed for solubilisationbeforeoralfeedingtoanimalsusinggavageat400mg/kgbodyweight[14].

AcuteToxicityStudy: Acutetoxicitystudyofsilica was determined by up-and-down method [15],intraperitonealadministrationofsilicaatLD$_{50}$(lethal dose) at 200mg/kg, and acute toxicity test of*Zizyphusmauritiana*extractswasdeterminedbyusingguidelinesofOrganizationforEconomicCorporationDevelopment(OECD)[15].Ratswereorally

administered daily with extracts of *Zizyphusmauritiana*and observed for the toxicity signs likemortality,lossofbodyweightandbehaviouralc hangesand obtainedLD_{50}value at 4000mg/kg.

Experimental Design: Thirty six adult male ratswere divided into six groups of six animals each.The animals were administered silica at the dose of20mg/kg ($1/10^{th}$ of the LD_{50} value, solubilised inphosphatebufferedsaline,0.1M,pH7.0),intraperit oneallyfollowedbyoralfeedingof*Zizyphusmauritia na*extracts at 400 mg/kg ($1/10^{th}$oftheLD$_{50}$value)for21days.

BiochemicalAnalysis:

Determination of Serum Alanine TransaminaseandAspartateTransaminase:S erumalaninetransaminase(ALT)andaspartatetra nsaminase(AST) were analyzed by the method of Reitman(1957)[16].

Determination of Serum Alkaline Phosphatase: Alkalinephosphatase(ALP)withit soptimumpHin the alkaline range liberates inorganic phosphate;ALPwas measured bythemethod of King*et al.,*[16].

Determination of Serum Lactate Dehydrogenase: Activity of oxidoreductase, that requires NAD^+ or$NADP^+$ as coenzyme was determined by measuringtherateofchangeofNAD^+insystemfoll owedby

Group1: Theanimalswerenotgivenanytreatment(n ormalcontrol).

Group 2: The animals received only silica for 21days(positivecontrol).

Group 3: The animals received silica with extractofroot bark (root preventive).

Group4: Theanimalsreceivedsilicawithsimultane ousextractofstembark(stempreventive).

Group 5: The animals received silica withsimultaneous extract of leaves (leaves preventive).**Group6:**Theanimalsreceivedonlypho sphatebufferedsaline (0.1M, pH7.0)(vehiclecontrol).

The doses were given to animals at particular timescheduledailybefore9AM from0 to 21 days.

Collection of Blood and Serum Samples: At theend of the experimental schedule, the animals weregivendeepdi-ethyl-ether(Merck)anaesthesia.After anaesthesia, blood was immediately collectedfromretroorbitalplexusthroughcapillaryt ube.The blood was allowed to clot for 30 min at roomtemperaturefollowedbycentrifugationusingsi mple table-top centrifuge (Remi CM 12 Plus) at1500rpmfor10mintoobtaintheserum.Thecolourl esssamplesserumstoredat-80^{0}Cuntil use. Blood samples were preserved at -40 ^{0}Cuntilanalysis.

BodyWeightMeasurement: Bodyweightofeveryr atineachgroupwascarefullyrecordedbefore initiation of experiment and at the end of theexperimental schedule and just before withdrawingthebloodfromanimals(EssaeTeraoka Ltd.FB200).production of lactate [17].

DeterminationofSerumCreatinine: Serumcrea tinineestimationwasmeasuredbyalkalinepicrate method[18].

DeterminationofBloodUrea: Bloodureaconcen trationwasdeterminedbyNesslerisationmethod bythe method ofCleon (1942) [19].

Uric Acid Level in Serum: Uric acid in the proteinfreefiltrateofserumwasmadetoreactwithp hosphotungsticacidreagent,inthepresenceofalkal ine medium to form blue coloured complex bythe method of Martinek(1965)[20].

TNF-αandIL-6: Levelofpro-inflammatorycytokines including TNF-α and IL-6 in serum wasevaluatedusingcommerciallyavailablemulti-analyte ELISA kit (Qiagen). Absorbance was readat 450 nm using Thermo electron Corp. 358 ELISAplatereader.

Determination of Total Bilirubin: Total Bilirubin(TB) in serum was measured by Malloy and Evylinmethod(1937).Bilirubinreactswithdiazoti zedsulphanilic acid to form purple colourazobilirubin.The intensity of the purple colour is proportional tothebilirubin concentration in theserum[21].

Haemoglobin Percentage: Haemoglobin level inbloodwasestimatedbySahli'sHaemoglobino-meter byacid haematinmethod[22].

Estimation of Blood Glucose level: Blood sugarwas estimated by AccurexGlu eco kit (Young, *etal*.,1975).

StatisticalAnalysis: Thedataobtainedwereexpressed as Mean ± Standard error of mean (n=6) andanalyzedwithone way ANOVA followedby Tukey Post hoc analysis comparison with eachgroup using GraphPad Prism version 5.0. P < 0.05wasconsidered as statisticallysignificant.

RESULTS: Body Weight Changes in Silica and*Zizyphusmauritiana*Treated Rats: Body weightofexperimentalanimalsbeforeandaftertheexperimental schedule has been presented in **Fig. 1**.Theresultsindicatethatratsfromthenormalcontrol group (Group 1) followed a normal patternofgrowthandattainedanormalweightgainreaching to 230 ± 8.12g from average 205 ± 7.13over 3 weeks. Animalsin positive control (Group2)sufferedgrowthretardationandhadasignificantly (P<0.05)lowerweightthanothergroups. Among the treated groups (Group 3, 4, 5),treatmentofanimalsbyextractsofstembark(Group4)wasmoresignificantwithrespecttoweight gain as compared to treatment with extractsofroots (Group 3)and leaves(Group5).

The body weight of treated group was quite near tothe group 1 animals *i.e.* a group where animals didnot receive any treatment as compared to group 2animals (cirrhosis positive control group rats).
Nosignificantchangewasobservedinvehiclecontrol (Group6)ascomparedwiththenormalcontrolgroup of rats'w. r. ttheweightgain.

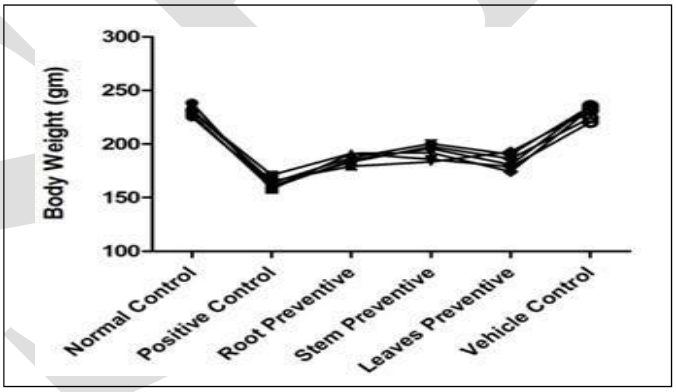
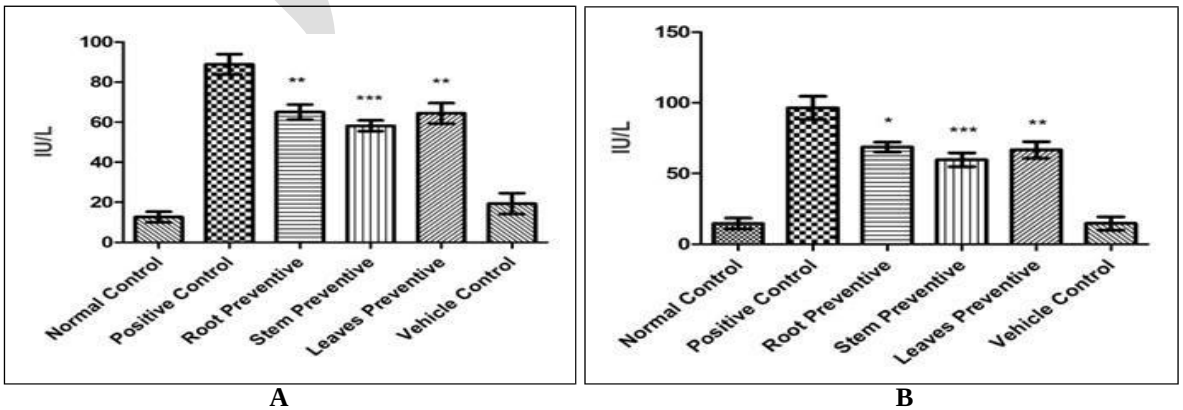


FIG.1:EFFECTOFEXTRACTSOF*Z.MAURITIANA* ONTHEBODYWEIGHTINSILICA TREATEDRATS

EffectonSerumAlanineTransaminaseandAspartateTransaminaseActivity:Fig.2a,2bshowsthatinsilicatreatedrats,increaseintheenzymatic activities of ALT,AST was significantascomparedtothecontrolgroup.Oraladministration ofextractsof root and stem bark,andextractofleavesof*Zizyphusmauritiana*significantly reversed their level towardsnormal.The dose of three different extracts revealed moresignificant therapeutic effectiveness as compared topositivecontrol.Thereisnosignificantchangeobserved in vehicle control group when comparedwithnormal groupof rats.



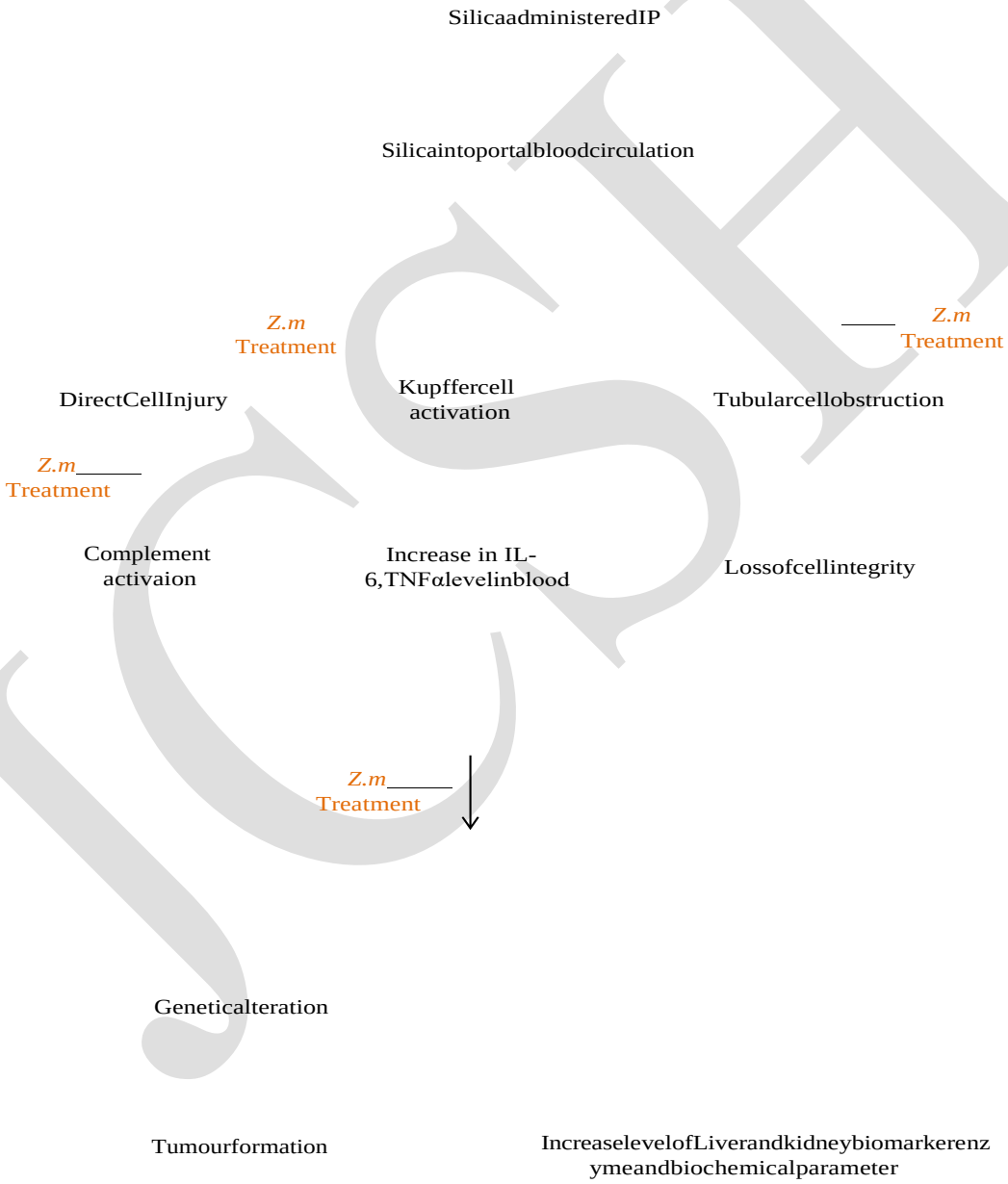
A **B**

FIG.2:EFFECTOFTREATMENTWITH*Z.MAURITIANA*EXTRACTSONALT(A)ANDAST(B)SERUMENZYMEACTIVITY OF SILICA TREATED RATS

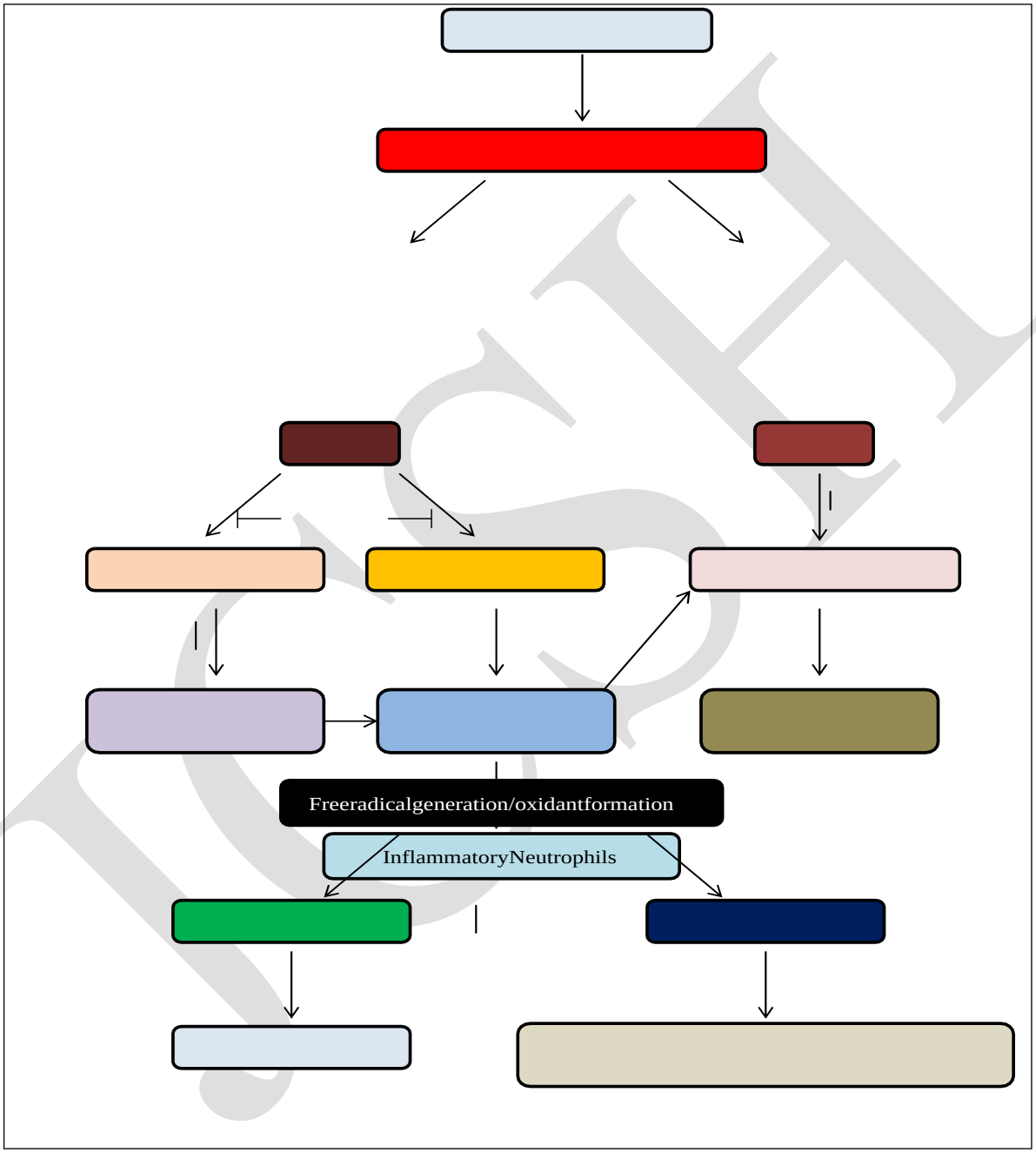
DataareexpressedinMeans±S.E.M.P<0.001comparedwiththenormalgroup.*P<0.05,**P<0.01and***P<0.001comparedwithpositive controlgroup.

DISCUSSION: Silicaisoneoftheabundantmineralsfoundontheearthwithvarieduse.Workingwithexposuretosilicaincreasestherateof silica induced adverse health effects. The presentstudy focuses on the preventative measure to silicainduced toxicity. The plant in this study is a folkmedicineusedsinceagestocurethediseases.Studieshavedemonstratedthatsilicainducedtoxicitymainlyaffects liverand kidneys.

Activities of serum amino transferases are normallyconsideredastoxicitymarkersinhepatotoxicitystudieseveninsilicosis.Ithasbeendemonstrated



thatintraperitoneallyadministeredsilicareachesthe liver through portal vein circulation and getsdepositedinliver,rupturingthehepaticcellmembranes leading to the release of the enzymesfrom the cytoplasm into the blood circulation [4]. Asignificant rise in the level of serum ALT and ASTinpresentcaseingroup2animalsmaybeduetothe ruptured hepatic cells. The present study alsorevealed an increase the level of ALP in rats treatedwithsilica,whichisagainanindicationofobstructivedamagein thehepatictissue[23].



:ASCHEMATICDIAGRAMMATICREPRESENTATIONSHOWINGPROTECTINGROLEOF*ZIZYPHUS MAURITIANA*INSILICAINDUCEDTOXICITY

Compared to the control groups, animals treated with silica had higher levels of creatinine, urea, and uric acid in their serum. This could be because the kidneys are damaged and their functions are disrupted, leading to dystrophic changes in the kidney tissues. Consequently, the excretion of these substances drops, and the serum content of these 24 is elevated. The serum lactate dehydrogenase activity of rats treated with silica increased significantly, which is another indicator of injury and necrosis in the kidney and liver tissues.

When LDH appears outside of cells, it can be an indicator of cell necrosis or an organ malfunction

25. Free radicals may be produced by silica, which in turn induces cytotoxicity in the cells of the liver. The local macrophages, which are non-parenchymal cells called kuppfer cells (KC), are crucial in defense because they phagocytose invading particles.
The generation of pro-inflammatory cytokines by activated KCs is also crucial for the maintenance of liver injury. Our findings suggest that silica exposure likely triggers KCs to secrete TNF-α and IL-6 into the bloodstream. Hepatic cells are negatively impacted by certain bioactive chemicals.

We found that the positive control group had higher blood levels of TNF-α and IL-6 compared to the normal healthy group, which might indicate that inflammation may have happened because macrophages were activated to produce pro-inflammatory cytokines after internalizing silica. Silica does not influence blood sugar, TB, or hemoglobin levels, as shown by the lack of statistically significant changes in both the positive control and Z. mauritiana extract treatment groups 26. Because it prevents bilirubin from being conjugated with glucuronides in the liver's smooth endoplasmic reticulum, a number of studies have shown that high doses of silica or excessive exposure to silica raise bilirubin levels 27. By blocking the free radical assault on membranes, Z. mauritiana extracts may be significantly reducing the peroxidation of lipids in these components. Several polyphenolic chemicals have been found in this plant in previous phytochemical investigations. Research has shown that this plant's bark, roots, stems, and leaves contain a plethora of secondary metabolites, alkaloids, and flavonoids (28).
Polyphenols help prevent cell damage by scavenging free radicals. The anti-reactive oxygen species 29 properties of these chemicals are encouraging. Compared to animals given extracts of root bark and leaves, those given stem bark were better able to protect themselves from silica's negative effects. Because Z. mauritiana contains several phenolic and flavonoid components with -H and -OH groups, its extracts have the ability to prevent and treat disease. These groups bind the free radical and prevent reactive oxygen species (ROS) from being formed when they are present.

The ascorbic acid found in abundance in Z. mauritiana leaves also aids in neutralizing free radicals. 12. In cases when male reproductive organ illnesses are caused by silica-induced toxicity, ascorbic acid plays a significant role in curing these conditions (31). The current research shows that biochemical parameters including creatinine, urea, and uric acid, as well as the liver and kidney biomarker enzymes ALT, AST, ALP, and LDH, are substantially reduced after oral administration of stem, leaf, and root extracts. Polyphenolic substances prevent the nitrosation of cells in the liver and kidneys, according to earlier research 32.
When exposed to silica on a regular basis, IL-6 levels rise, which in turn protects cells from free radical damage. It is known that the cyclooxygenase (COX) 34 enzyme may be inhibited by the phytochemical content of stem bark, root bark, and leaves. Inflammatory prostaglandins (PGE2) are produced by the COX-2 type of this enzyme; IL-6 is strongly induced by this PGE2. When exposed to xenobiotics, oxidative stress raises levels of TNF-α, a key pro-inflammatory cytokine. Because Z. mauritiana contains 36 flavonoids that inhibit the NF-kβ, the groups treated with Z. mauritiana (stem, root, and leaves) showed a substantial drop in TNF-α levels. Multiple studies have shown that the flavonoid quercetin inhibits NF-kβ1 gene production by reducing phosphorylation of Ik-βα and Ik-ỤỤ 37, which in turn has anti-inflammatory effects.

CONCLUSION: Zizyphusmauritiana aqueous extracts have shown promising protective effects against silica-induced toxicity.
The antioxidant properties of Z. mauritiana may be assisting in cellular homeostasis. Based on the results of this study, Z. mauritiana may have an anti-inflammatory impact, which might pave the way for new neutral-pharmaceutical compounds to treat inflammatory illnesses.

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