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AMORPHOUS SILICA MEDIATED CONCENTRATION AND DURATION INDEPENDENT MODULATIONS IN LIVER FUNCTIONS

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ABSTRACT:

Role of silica in humans and animals is being discussed for a good number of years in the past however silica recalls extensive research over its bioactivity after its use and essentiality being established from animal development to cell culture media. In the present work male albino rats were treated with 10, 20, 30, 40, 60 and 80 mg/kg BW of silica in the form of SiO_2 for acute and chronic study. The animals were given above-mentioned doses for one, seven, fourteen and twenty one days. The serum parameters of liver functions were assessed viz aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase.

INTRODUCTION:

There is requirement of some ions in very little amount and are called as trace elements viz. iron, copper, manganese, molybdenum, zinc selenium, vanadium [1] while many of them are being realized in recent years as the cell specific demands are being established [2]. Silica is one of such elements which being studied for its role in animals.

Silica

There is continuing debate as to whether there should be an RDI for silica in humans. Silica is omnipresent in drinking water and is found in surface and well water in the range of 1 - 100 mg/L. However, silica is not listed in the Primary or the secondary Drinking Water Standards. There are number of studies with compelling results suggesting the essentiality of silicon, delivered as silica, for humans [3].

As reviewed from Martin [3] a functional role for silicon has yet to be identified but clearly it is feasible and likely. Silicon is known to be required by chicks and rats for growth and skeletal development. For example, inducing silicon deficiency produces profound results including deformities in skull and peripheral bones, poorly formed joints, reduced mineral contents of cartilage, collagen, and disruption of mineral balance in the femur and vertebrae. The obvious

effect of silicon deficiency on bone supports the notion that it is critical for bone formation as shown in studies with chickens and rats [4,5,6,7]. Chicks fed silicon-deficient diets also showed structural abnormalities in the skull and long bones such as the femur [8]. More in depth studies using rats deprived of silicon showed decreased bone hydroxyproline levels and inhibited alkaline and acid phosphatases [9]. Regarding the former, silicon has been shown to contribute to prolylhydrolase activity necessary for normal collagen formation [8]. Silica enhances and maintains articular cartilage and connective tissue due to interaction of silicon with glycosaminoglycan formation, a structural building block of these tissues. Silicon is also a constituent of enzyme(s) involved in bone matrix formation suggesting a role in bone calcification. Clearly, silicon is localized in sites of active bone growth supporting a role for dietary silica. Silicon has also been suggested to exert a protective role in atherosclerosis, in part, due to maintenance of blood vessels may because the increased silica consumption reduces the incidence and severity of atherosclerosis presumably through its effects on blood vessel-associated glycosaminoglycan and collagen integrity and function.[10] . Silica which is food grade is also reported to be hypocholesterolemic [11] and hepatoprotective against hepatotoxin CCl_4 [12,13] . Silica is also thought to be beneficial in Alzheimer's disease because silicon can interact with aluminum and prevent aluminum toxicity often associated with Alzhemier's disease [14] . This protective effect has also been noted in humans where dietary silicon protected against aluminum accumulation and presumably neurodegenerative effects [15]. Collectively, evidence supports a protective role for silicon in maintaining bone health, cartilage and connective tissue structure, prevention of toxicity to the brain, and maintenance of blood vessel integrity [3].

Liver

Any injury to liver that results in cytolysis and necrosis causes the liberation of various enzymes. The measurement of these hepatic enzymes in the serum is used to assess the extent of liver damage and to differentiate hepatocellualr (functional) from obstructive (mechanical) disease. The most common enzymes assayed in hepatobiliary disease include ALP and the aminotransferases. In the following text, enzymes which are considered as the markers of hepatocyte damage or liver functions are reviewed [16,17,18,19]

Aminotransferases

Among the most sensitive and widely used of these liver enzymes are the aminotransferases (AST or SGOT). These enzymes are normally contained within liver cells. If the liver is injured, the liver cells spill the enzymes into blood, raising the enzyme levels in the blood and signaling the liver damage.

Aspartate Aminotransferase (AST) is classified under transferases. It transfers amino group between aspartate and ά-ketoglutarate and ά-keto acids where Pyridoxal phosphate functions as co-enzyme. The transamination reaction is important intermediary metabolism and is involved in the synthesis and degradation of amino acids.

The keto acids formed are oxidized by TCA cycle to provide source of energy [18] reviewed the functional and clinical significance of AST.

The clinical use of AST is confined mainly to the evaluation of myocardial infarcation, hepato cellular disorders and skeletal muscle movement. The enzyme activity is frequently seen in pulmonary embolism. Following congestive heart failure, AST levels are increased probably reflecting liver involvement due to inadequate blood supply to that organ. AST levels are highest in acute hepatocellular disorders. In viral hepatitis the enzyme levels may reach 100 times. In Cirrhosis may be only 4 times. In skeletal muscle disorders such as the muscular dystrophies and inflammatory conditions the levels are also increased. AST in humans exists as two isozymes one in cytosol and another in mitochondria.

Cytosolic form is predominantly found in serum. In disorders like cirrhosis the mitochondrial form may be markedly increased. Isozyme analysis of AST is not routinely performed in the clinical laboratory.

Alanine aminotransferase (ALT) is a transferase with enzymatic activity similar to that of AST. Specifically it catalyses transfer of an amino group from alanine to ά-Ketoglutarate, forming glutamate and pyruvate. Though it is present in many tissues it is rich in liver and is considered as the liver specific enzyme. Therefore it is used to evaluate the hepatic disorders/acute inflammation in liver. High levels of elevations are noted in hepatocyte disorders than in extra or intrahepatic obstructive disorders. In acute inflammatory conditions of the liver, ALT elevations will frequently be higher than observed in AST. The elevations tend to remain longer since ALT's half-life in serum is high. Cardiac tissue contains small amount of ALT, but it does not affect serum levels in cardiac failures, unless sufficient liver damage has occurred. AST and ALT both are required for the diagnosis of liver involvement with myocardial injury in case of human.

Alkaline phosphatase (ALP) -

Alkaline phosphatase in serum is a group of non-specific enzymes that hydrolyzes aliphatic, aromatic and heterocyclic phosphoric acid esters with pH optima between 9 to 10. It is derived from several different tissues that include; liver, intestine, bones, placenta, etc. Liver clears serum alkaline phosphatase. Both hepatocellular disease and bile duct abnormalities, therefore affects the serum ALP activity in the early obstructive disease and its levels may increase even before bilirubin increases. It is increased considerably in inflammatory diseases and metastasis carcinoma of the liver, biliary cirrhosis cholangiolytic hepatitis. Their isoenzymes help to describe the origin of tissues, e.g. in human increase in α-isoenzyme points to liver damage, β-isoenzyme points to increase in osteoblastic activity γ-isoenzyme to intestinal lesions. In hepatobiliary disorders ALP bands have been identified in regions of α_1 and α_2 globulins. α_2-isoenzyme is derived directly from liver cells and α_1 band represents the regulation of bile alkaline phosphatase, which is markedly elevated in metastatic carcinoma of liver, obstuctive jaundice and cholangitis. α_1 band is specific to viral hepatitis. In rat serum ossesous and intestinal isoenzyme are dominant and the food intake affects the proportion of intestinal alkaline phosphatase in plasma [20]. However in rat it can be used to evaluate the clearance of alkaline phosphatase since in normal liver alkaline phosphatase is not present [21] and appears in pathological conditions may be for clearance.

Animals

For the present project only male animals were used to avoid any hormonal changes that occur in female rats since the experiment was designed for chronic studies as well.

Dose selection:

A dose equivalent to 2,500 mg/kg bw/day silicon dioxide, or 1,165 mg silicon/kg BW/day was regarded as a NOAEL (No Adversse Effect Level) [22, 23] . Besides there are no recommended intakes or toxicity levels set for the silica in either humans or animals, wide dose range from 10 mg/kg BW to 80 mg/kg BW was selected with 10, 20, 30, 40, 60 and 80 mg/kg BW.

Duration selection

Single dose associated immediate responses are helpful in deciding the doses and analyzing the toxicity. Silica toxicity that had been studied so far [24, 25, 26] has shown to accumulate silica. Hence an acute study to understand immediate responses and prolonged treatments for understanding of chronic effects resulting from accumulation of silica and associated toxicity were carried in the present project. For the same the selected doses were given for one, seven, fourteen and twenty one days.

Selection of oral route to deliver silica:

Silica related toxicity studies are associated with exposure to particulate matter leading to silicosis. Therefore there is need to study oral treatment associated toxicity. Many authors have stated that with very large doses there is no apparent toxicity [27] or some of them who have observed the toxicity they have mentioned that there are lesions in different organs [28] however there is no analysis of such lesions therefore oral toxicity associated alterations needed to be studied. Additionally as stated before, the study also aimed to study toxicity associated with dietary silica. Hence the oral route was selected for these studies.

MATERIAL AND METHODS:

Animals

Male albino rats used in the experiments were bred in the Departmental animal house (Reg. No. 233/CPCSEA for breeding and maintenance of rats and mice) of Zoology Department, Shivaji University, Kolhapur, India. The animals were selected of age 90-100 days and weighed 130-140 gm were maintained in standard of conditions of temperature, humidity and 12 hr light/dark cycles. The animals were fed with standard laboratory feed (Amrit feeds, Sangli, MS, India) and water *ad libitum*. The experimental protocol was approved by departmental animal ethics committee and the experiments were carried out according to guideline of 'Committee for prevention and control of scientific experimentation on animals (CPCSEA)

Chemicals:

SiO_2 in the form of amorphous powder was purchased from local trader (Nilkanth Minechem) at Kolhapur. The same samples were used throughout the experiment.

The Best Quality chemicals were used to carry the present work. Pathological diagnostic kits were purchased from AGAPPE Diagnostics, India. Other chemicals used were of Analytical grades.

Experimental Protocol :

The animals were divided into 7 different groups and were given the silica in following manner.

Group 1: The animals were not given any treatment

Group 2: The animals received 10 mg/kg body weight silica

Group 3: The animals received 20 mg/kg body weight silica

Group 4: The animals received 30 mg/kg body weight silica

Group 5: The animals received 40 mg/kg body weight silica

Group 6: The animals received 60 mg/kg body weight silica

Group 7: The animals received 80 mg/kg body weight silica

The above schedule was repeated for one, seven, fourteen and twenty one days to understand the acute as well as chronic effects. All treatments during the experiment were given between 08.00 a.m. to 10.00 a.m. and the animals were also sacrificed between the same times on the next day. The doses were given using gastric tube to ensure the desired quantity.

Collection of serum -

At the end of the experimental schedules described above, the animals were sacrificed giving deep ether anesthesia. Immediately after killing the blood was aspirated from the left ventricle with the sterile syringes and was allowed to clot at room temperature in Test-Tubes. On clotting the Tubes were centrifuged using simple table-top centrifuge to obtain the serum samples. The colorless serum samples were stored at 10^0 C until use.

RESULTS:

1. Serum Aspartate Aminotransferases:

The results of Serum Aspartate Aminotransferases are given in the Table1

One day treatment -

The control group exhibited 30.38 ± 1.96 units/dl AST activity. The silica treatment of 10 to 30 mg doses elevated the levels by 1.67, 3.33 and 1.33 folds respectively. The 40 mg dose normalized the levels, but again there was decrease by 33.33%

with 60 mg and 1.33fold increase with 80 mg dose of SiO_2.

As far as single dose effect within 24 hours is considered, these alterations may be having an immediate impact on release of the enzyme molecules from some of the hepatocytes. It is known that Ca^+ influx by silica activation leads to cell injury which is affected through calcium channels of plasma membrane [29]. In present case SiO2 has altered it significantly indicating the hepatocytes may have damaged their plasma membrane to release AST activities in serum by Ca^+ influx. Silica crystals are absorbed through gastrointestinal tract and reach hepatocytes/liver as orthosilicic acid. Hence it may be possible that it is reaching liver through blood where it exists as silicic acid [30] . AST is mainly confined to the evaluation of the myocardial infarction, hepatocellular disorders, skeletal muscle movement and pulmonary embolism [18]. In present case the silica is orally administered and possibility of reaching it on absorption in liver than in any other tissue is high therefore influence or shifts in metabolism seems to be influenced in different doses must be taken as impact on liver metabolism. SiO_2, where 10, 20 and 30 mg elevated AST activity while, but 40 mg/kg BW did not influenced the activity and 60 mg inhibited the activity while 80 mg again elevated the activity exhibits concentration independent influence, may be involving some unknown mechanism of action. However for the reason given above [18] the activities are indicators of liver alterations rather than any other organ.

Seven days Treatment -

The normal rats exhibited 31.01 ± 1.74 units of AST activity per dl of the serum. All the doses of silica resulted in decreases in the levels of AST. The 10 to 80 mg doses exhibited respectively 40.00, 37.50, 50.00, 75.00, 71.25 and 73.75 % decrease.

While a week prolong treatment (seven doses, one per day) of SiO2 showed different responses, than were observed with single dose. Seven doses given are dose per day inhibited the activity (highly significantly) with 40, 60 and 80 mg /kg BW doses.

Fourteen days Treatment -

The normal rats exhibited 33.76 ± 2.17 units of AST activity per dl of the serum. With the silica treatment the lower doses, of 10 and 20 mg/kg BW elevated AST levels by 1.3 and 1.2 folds respectively. With the 30 mg dose the levels were normalized. Further doses of 40 and 60 mg /kg BW silica decreased the AST levels by 10 and 20 %. Eighty mg dose exhibited an increase of 1.2 fold.

This trend was different from the trend observed with single dose or 7 days treatment, which showed instead of inhibition, stimulation (marginal) of AST activities with lower doses (10 and 20 mg/kg BW).

Twenty-One days Treatment -

The normal rats serum reported 38.82 ± 2.50 units of AST activity per dl. Ten , 20 and 30 mg/kg BW silica doses resulted in the increases by 1.09, 1.09, and 1.13 folds respectively. The 40 mg/kg BW dose showed a slight decrease by 4.35% but again the levels were raised by 1.04 and 1.13 folds with 60 and 80 mg silica respectively.

The stimulation of AST activities by 10 and 20 mg SiO_2 was shown by all the treatments of SiO_2 except 7 days treatment. Inhibition of AST activities on seven days treatment is not observed in any of the durations studied. SiO2 had activated the calcium influx through dmaged plasma membrane leading to release of AST in serum [29] .

2. Serum Alanine Aminotransferases:

The Observations of Serum Alanine Aminotransferases are given in the Table 2.

One day treatment -

The control group exhibited 30.26±1.31 units of ALT activity. With 10 and 20 mg silica, there wasn't any deviation observed. However 30, 40 and 60 mg/kg BW doses decreased the levels by 33.37, 66.68, and 50.00%, which were again raised with 80 mg/kg BW dose by 1.33 fold.

Acute SiO_2 treatment showed a different trend in ALT than AST, where 30 mg to 60 mg /kg BW single doses showed inhibition but 60 and 80 mg Vajrabhrak maintained normal activity, while in case of SiO_2 10 and 20 mg maintained ALT activity. The single doses of SiO_2 may have inhibited the release of ALT in serum or rapid clearance of ALT from serum altering the half life of ALT in serum or inhibiting their activity partially. However highest doses of 80 mg/kg BW of SiO_2 seem to release ALT in serum indicating stress. Acute SiO_2 treatment showed a different trend in ALT than AST, where 30 mg to 60 mg /kg BW single doses showed inhibition but 60 and 80 mg Vajrabhrak maintained normal activity, while in case of SiO_2 10 and 20 mg maintained ALT activity. SiO_2 (7 doses of all the doses used) inhibited the activity. However 10 and 20 mg/kg BW doses did not alter the ALT activities showing no harm. This is immediate response and chances of immediate stress with high doses are possible.

Seven days Treatment -

The normal rat serum exhibited 34.44 ± 2.22 units of ALT activity. The SiO_2 treatment maintained the levels below the normal with all the given doses. Thus 10 to 80 mg doses exhibited a decrease by 21.58, 8.84, 21.58, 50.99, 78.43, 49.03 % respectively.

SiO_2 (7 doses of all the doses used) inhibited or depleted ALT activity. The reductions in the levels of ALT were nearly the same with 10, 20 and 30 mg/kg BW doses. However all higher doses inhibited the release of ALT from hepatocytes or inflammatory activities or conjugatedly inhibiting the activities in serum by its metabolic products or by accelerating the clearance of the ALT molecules. These results are indicators of influence of Silica compounds on ALT. When it is in free form seven doses inhibit ALT activity in serum

Fourteen days Treatment -

The normal rat serum exhibited 33.76±2.18 units of ALT activity. Ten, 20 and 30 mg silica treatments showed increases of 1.3, 1.2 and 1.1 folds respectively. There was a decrease of 10% with 40 mg/ kg BW of silica. The 60 mg dose of silica normalized the levels but further dose of 80 mg increased the levels of ALT by 1.3 folds. This 2 weeks treatment of SiO_2 showed the maintenance or marginal increase in ALT activity. At this interval/doses of treatment received, seems to equilibrate SiO_2 influenced metabolic conditions which are responsible for serum ALT levels where marginally high ALT levels with 10, 20 and 80 mg/kg BW doses were observed

Twenty-One days Treatment -

The untreated group of animals exhibited 40.23 ± 2.85 units of serum ALT.

The SiO_2 doses of 10 and 20 mg/kg BW maintained the levels below the control showing decreased levels by 36.33, 87.76 % respectively , and 30 as well as 40 mg doses exhibited a slight decrease by 2.05 and 4.05 % respectively. The higher doses caused the remarkable increases by 2.01 and 3.16 folds. Silica as oxide inhibited the ALT activity in early all doses may be the metabolites of SiO_2 inhibited release of ALT in serum or rapid clearance of ALT in serum. Steady increase in ALT activities in serum indicated the possible accumulation of ALT. Therefore the inhibition may be because of any other reason than the clearance of ALT.

Since ALT levels in serum are indicators of hepatic disorders/inflammatory processes [18] in present condition both of these possibilities exist since oral route allows the accumulation of silica in liver primarily [31] and metabolize it. Silica associated inflammations are known. Prolonged treatments of SiO_2 may be accumulating Silica in various regions as silica is known to be accumulated in different compartments of cells [31] and possibility of associated inflammatory activities can't be eliminated. Besides slow clearance of serum ALT [18] also exists.

3. Serum Alkaline phosphatase:

The Results of Serum Alanine Aminotransferases are given in the Table III:03 and Figure III:03.

One day treatment -

Alkaline phosphatase activity in the serum of normal rat was observed to be 157.5± 10.16 units/dl. Ten and 40 mg silica treatment enhanced ALP by 1.19, 1.43, 1.43 and 1.67 folds respectively. The two higher doses of 60 and 80 mg showed a marginal decrease by 4.76 and 6.03 % respectively.

The results indicated that SiO_2 stimulated ALP activity with 10 to 40 mg/kg BW doses but not with higher doses where ALP activity except 10 mg dose which did not alter the activity. The results obtained are the immediate effects of single dose. ALP is contributed by other organs like bone and also by liver disorders [18]. In present condition treatment is in single dose and route is of liver therefore it is primarily concentrated in liver in distribution since ALP is cleared through liver [20] stimulation observed may be inhibition of ALP clearance through bile may have conjugatedly hindered by high concentration of SiO_2.

Seven days Treatment -

The levels of ALP activity in the serum of normal rat were observed to be 120±7.74 units/dl. All doses of silica exhibited decreases in the levels (except 20 mg dose where it was unchanged). Ten to 80 mg doses reported 66.67, 33.33, 66.67, 83.33 and 66.67 % decreases respectively.

Seven days SiO2 treatment indicated cyclic reactions which were stimulated with 10 mg/kg BW and maintained with 20 mg /kg BW and though appear depleted by 80 mg/kg BW as compared to trend it should have been continued to deplete but it initiated stimulation again. This was not true with SiO_2 all doses inhibited ALP. These results indicated that SiO_2 may be influencing production of ALP rather than rapid clearance through liver as SiO_2 is known to affect heart [32] and bone marrow cells [33]

Fourteen days Treatment -

Normal rat exhibited 105 ± 6.77 units/dl of serum ALP. All doses of Silica exhibited decreases in the levels. The designed doses of 10 to 80 mg/kg BW Silica reported 14.29, 7.14, 14.29, 21.43, 14.29 and 28.57 % decreases respectively.

SiO_2 treatment of all the doses of SiO_2 inhibited marginally the activities of ALP. The inhibition was highest with 20 mg/kg BW dose and lowest with 80 mg/kg BW dose.

Twenty-One days Treatment -

The ALP activity in normal rat was 150.0±10.16 units/dl. Ten to 80 mg/kg BW silica doses showed 1.31, 1.22, 1.13, 1.22, 1.05 and 1.39 fold increases respectively. Results indicated that during twenty one doses SiO_2 might have accumulated in organs that is/are source/s of ALP and concentrations of SiO_2 may be in such condition where free silica ions are available to influence ALP release in serum.

The results obtained are of varying type and a clear dose or time dependent trend is absent. Wherein there are evidences of silica deficiency related abnormalities stressing its need as a trace element, [22] and supposed to be used as biomaterials Biomedicine [34] there are evidence of using silica nano-particles as hepatotoxins [35]. However the research postulating probable use of silica nano-particles as hepatotoxins also provided varying results with varying concentrations and particle sizes [35]. Recently many researchers have worked with the silica particles and reported interesting findings that are contradictory to each other. In one form (as hydride) silica has protected hepatocytes against CCl_4 mediated hepatotoxicity [12] however silica as nano-particles is referred as a hepatotoxin [35]. Wang et al (2011) [34] reported that exposure of SiO2 nanoparticles leaded to cellular morphological modifications, mitochondrial dysfunction, and oxidative stress as indicated by elevation of intracellular ROS and TBARS, as well as depletion of GSH, which triggers cell death in a dose-dependent manner in PC12 cells [34] . It is suggested that the size of silica particles contributes to the effects exerted by SiO2. [36]. The investigations carried to understand intercellular localization of silica NPs

[37, 36, 38, 39, 40] are not conclusive, rather contrasting. Hence the varying bioactivity of silica particles (in the form of SiO2 or NPs) may be a result of particle size, shape, chemical composition and possibly also depends upon cell and organ type being primarily exposed and distantly affected.

CONCLUSION:

Notwithstanding its limitations, this study indicates that there is a more specific need of understanding of nature and form of silica, before establishing RDI or requirement in the body. The work provides toxicological data on the effect of amorphous silica on liver functions of male albino rats. Liver being the first site for the biotransformation of xenobiotics, effect of any form of biomedicine or dietary supplement must be seen on the liver. A detail chemical and bioactivity study of the silica forms involving chemistry and biochemistry and probably biophysics is required to interpret and link all the sporadic results obtained from various research groups.

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Table 01: Silica (SiO_2) influenced alterations in serum aspartate aminotransferase activity of male albino rats (Values are expressed as units/dl

	One Day			Seven Days			Fourteen Days			Twenty-One Days		
Normal	30.38	±	1.96	31.01	±	1.74	33.76	±	2.17	38.82	±	2.50
$SiO_2$10	50.64	±	2.78 [c]	16.20	±	0.94 [c]	43.88	±	2.55 [c]	42.0	±	2.39
SiO_2 20	101.28	±	5.16 [c]	16.88	±	0.99 [c]	40.51	±	2.39 [c]	42.2	±	2.35
SiO_2 30	40.51	±	2.45 [c]	13.50	±	0.71 [c]	33.76	±	1.79	43.88	±	2.38 [a]
SiO_2 40	30.38	±	1.73	6.75	±	0.40 [c]	30.38	±	1.80 [a]	37.13	±	1.87
SiO_2 60	20.256	±	1.15 [c]	7.76	±	0.41 [c]	27.00	±	1.45 [c]	40.51	±	2.03
SiO_2 80	40.512	±	2.03 [c]	7.08	±	0.37 [c]	40.51	±	2.13 [c]	43.88	±	2.25 [a]

Values are mean ± SE of 6 animals p – values : a < 0.05, b < 0.01, c < 0.001 vs. normal

Table 2. Silica (SiO_2) influenced alterations in serum alanine aminotransferase activity of male albino rats (Values are expressed as units/dl)

	One Day			Seven Days			Fourteen Days			Twenty-One Days		
Normal	30.26	±	1.31	34.44	±	2.22	33.76	±	2.18	40.23	±	2.85
$SiO_2$10	29.90	±	1.15	27.01	±	1.66 [c]	43.89	±	2.69 [c]	25.61	±	1.73 [c]
SiO_2 20	30.25	±	1.07	31.40	±	1.99	40.51	±	2.56 [c]	23.13	±	0.34 [c]
SiO_2 30	20.17	±	0.69 [c]	27.01	±	1.52 [c]	37.14	±	2.09 [a]	39.41	±	2.43
SiO_2 40	10.08	±	0.35 [c]	16.88	±	0.99 [c]	30.38	±	1.79 [a]	38.42	±	2.48
SiO_2 60	15.13	±	0.53 [c]	17.43	±	0.39 [c]	33.76	±	1.79	80.78	±	4.70 [c]
SiO_2 80	40.34	±	1.39 [c]	17.56	±	0.93 [c]	43.89	±	2.32 [c]	127.08	±	7.39 [c]

Values are mean ± SE of 6 animals *p – values : a < 0.05, b < 0.01, c < 0.001 vs. normal*

Table 3. Silica (SiO_2) influenced alterations in serum alkaline phosphatase of male albino rats (Values are expressed as units/dl)

	One Day			Seven Days			Fourteen Days			Twenty-One Days		
Normal	157.50	±	10.16	120.00	±	7.74	105.00	±	6.77	150.00	±	10.16
$SiO_2$10	187.50	±	10.65 [b]	40.00	±	2.27 [c]	107.14	±	5.11	196.25	±	9.59 [c]
SiO_2 20	224.00	±	12.57 [c]	120.00	±	6.70	116.07	±	5.45 [a]	183.17	±	8.80 [c]
SiO_2 30	226.00	±	11.01 [c]	80.00	±	4.35 [c]	107.14	±	4.89	170.08	±	7.95 [a]
SiO_2 40	262.50	±	13.26 [c]	40.00	±	2.02 [c]	98.21	±	4.17	183.17	±	7.95 [c]
SiO_2 60	150.00	±	7.54	20.00	±	1.08 [c]	107.14	±	4.52	157.00	±	6.78
SiO_2 80	148.00	±	7.69	40.00	±	2.05 [c]	89.29	±	38.46 [b]	209.33	±	9.23 [c]

Values are mean ± SE of 6 animals *p – values : a < 0.05, b < 0.01, c < 0.001 vs. normal*