Sodium Thiosulfate Ameliorates Oxidative Stress and Preserves Renal Function in Ethylene Glycol-treated Rats

Rakesh Bijarnia and Andreas Pasch
Department of Nephrology and Hypertension, University Hospital Bern, Switzerland

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Introduction

The pathogenesis of renal calculi requires formation of crystals and their retention in the renal tissue. A series of reports have proposed that oxalate at higher concentrations causes oxidative stress in renal tissue. Thus, quenching oxidant burden appears to be a tangible way to combat the manifestations associated with renal calculi formation. Sodium thiosulfate (STS) reduces kidney stone formation in humans and minimizes calcium phosphate kidney stone formation in hypercalciuric rats. In the present study, we have focused on its antioxidant potential in *in vivo* & *in vitro* models.

Methodology

Wistar rats weighing 100-150 g were divided into following five groups-
Control: Normal food and water ad libitum for 28 days
Ethylene glycol (EG): EG (0.75%) was given in drinking water for 28 days to induce hyperoxaluria.
EG+ Sodium thiosulfate(STS): EG (0.75%) and STS (0.4 g/kg body weight) was given in drinking water for 28 days
EG+ sodium chloride(SC): EG (0.75%) and SC (0.4 g/kg body weight was given in drinking water for 28 days
EG+ sodium sulfate (SS): EG (0.75%) and SS (0.4 g/kg body weight) was given in drinking water for 28 days

After the treatment, renal functionality was studied by creatinine clearance. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were estimated in tissue and renal tissue histology was performed to analyze crystals deposition. Further, LLC-PK1 cells were exposed to free oxalate (1 mM) and load of reactive oxygen species (ROS) by using carboxy-H_2DFFDA dye and hydrogen peroxide (H_2O_2) scavenging ability were studied.

Results

Values in brackets are % increase (+) or % decrease (-) as compared to control group.
#p< 0.05, ##p < 0.01, ###p < 0.001: Indicates significant change in comparison to control group;
*p < 0.05,**p < 0.01, ***p < 0.001: Indicates significant change in comparison to EG group.

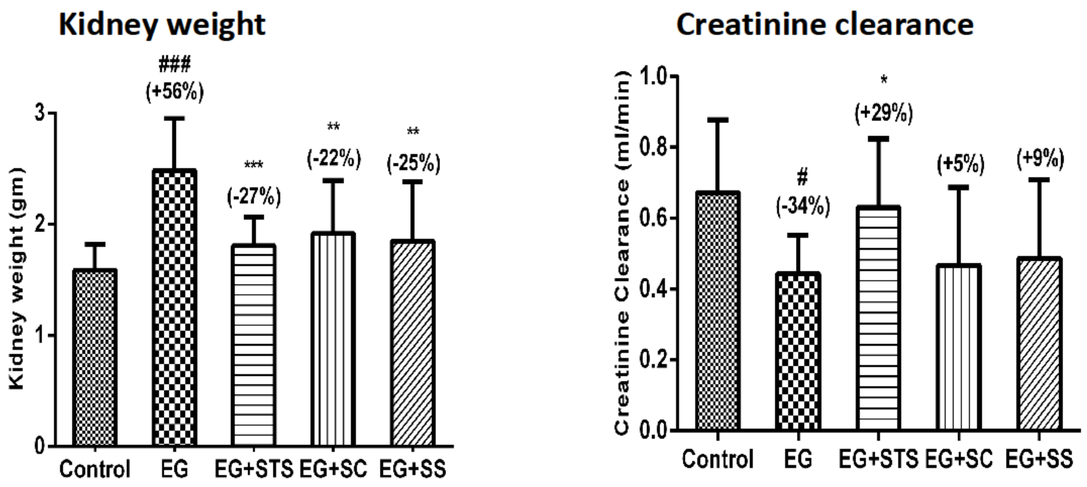
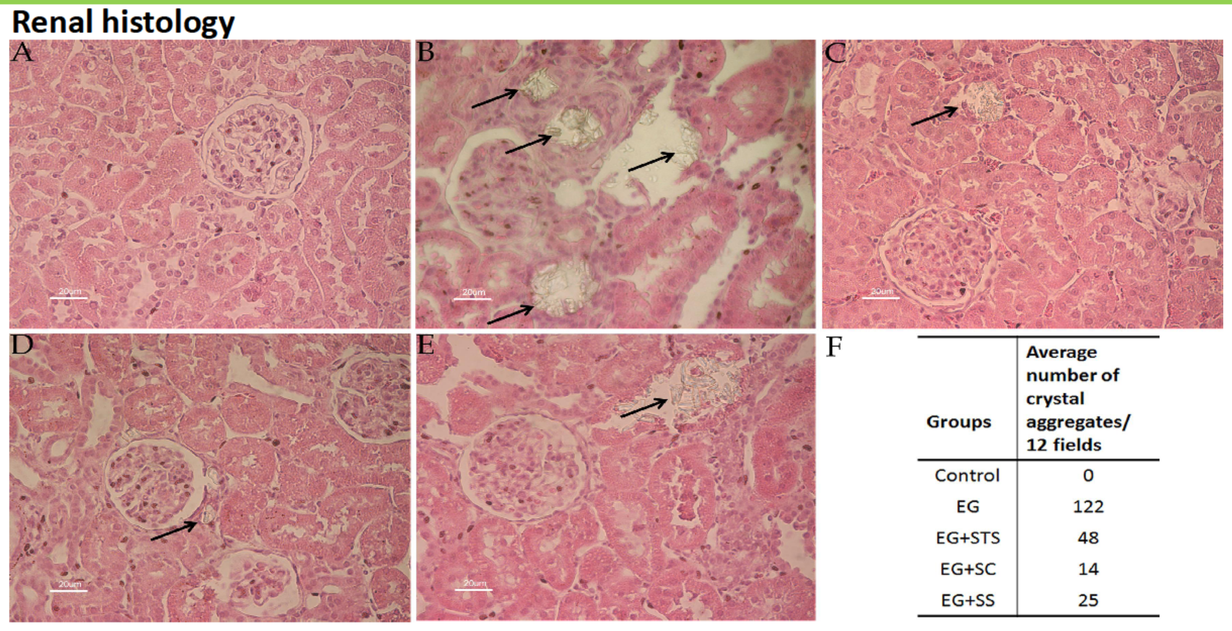
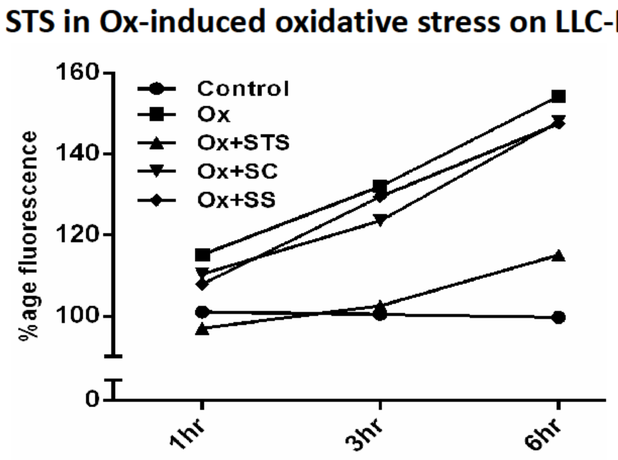


Figure 1: Treatment effects on kidney weight and renal function.
(A) Kidney weight was increased in the EG group, but remain near-normal in the EG+STS, EG+SC and EG+SS groups. (B) Creatinine clearance derived from 24-hour urine samples collected at 4 weeks showed preserved renal function in STS-treated animals.



Groups	Average number of crystal aggregates/ 12 fields
Control	0
EG	122
EG+STS	48
EG+SC	14
EG+SS	25

Figure 2: Representative hematoxylin- and eosin-stained kidney sections. (A) control, (B) EG-exposed, (C) EG+STS-exposed, (D) EG+SC-exposed, (E) EG+SS-exposed animals. Arrows indicate calcium oxalate crystal deposits. (F) Results of light microscopic quantification of crystal aggregates.



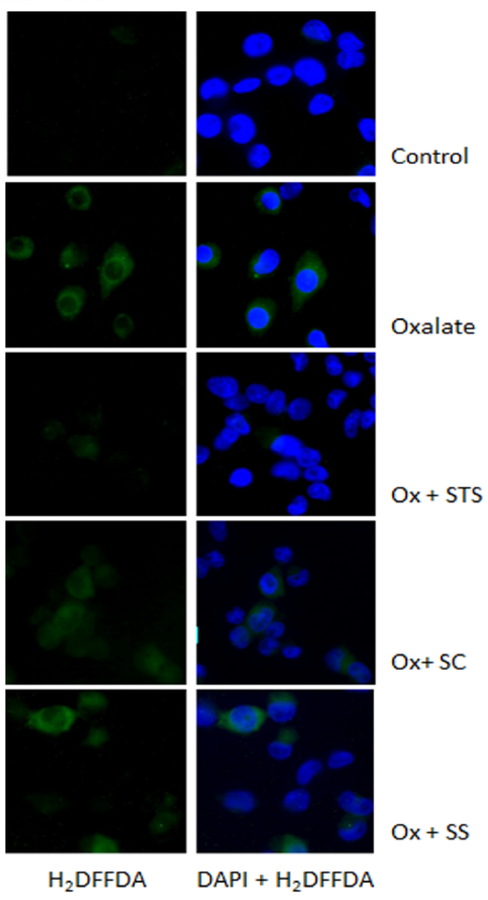


Figure 4: STS reduces intracellular oxidative stress.
Oxidative stress was induced by exposure of 1mM oxalate to proximal tubular LLC-PK1 cells and detected using the fluorescence dye H_2DFFDA. (A) Fluorescence changes in untreated LLC-PK1 cells (Control), oxalate (Ox) -exposed LLC-PK1 cells (1 mM), Ox+STS, Ox+SC and Ox+SS-exposed LLC-PK1 cells. STS largely protects LLC-PK1 cells against Ox-induced oxidative stress. (B) Dose-dependent protection by STS against Ox-induced ROS in LLC-PK1 cells. (C) H_2DFFDA- and DAPI-fluorescence imaging of LLC-PK1 cells. The absence of green fluorescence in Ox-exposed, STS-treated LLC-PK1 cells indicates the quenching of intracellular oxidative stress. Control = 100% in (A) and (B).

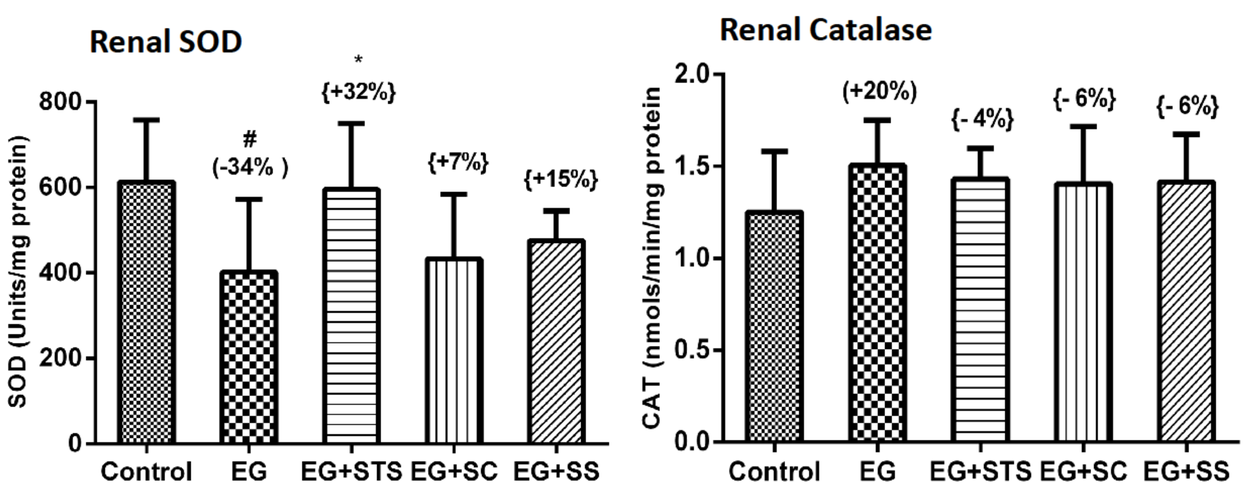


Figure 3: Activity of antioxidant enzymes. (A) SOD, and (B) CAT were determined in renal tissue . SOD was found to normalized in STS treated group.

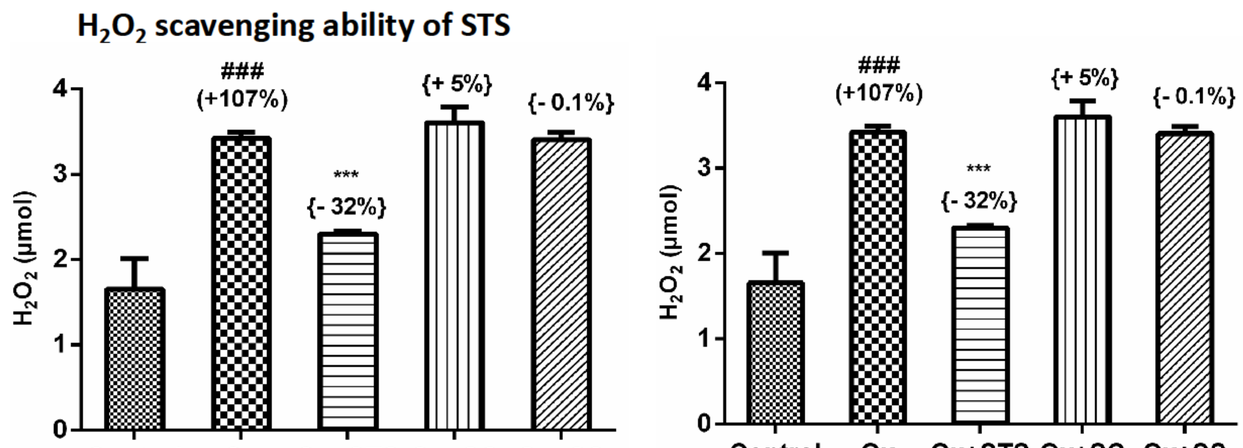


Figure 5: Intra- and extra-cellular quenching of H_2O_2 by STS.
(A) Ox-exposure of LLC-PK1 cells leads to the intracellular generation and accumulation of H_2O_2, STS-treatment reduced the amount of H_2O_2 released from the cells after 72 hours, whereas SC- and SS-treatment did not showed any H_2O_2 scavenging effect.(B) STS directly quenches H_2O_2 in aqueous solution.

Conclusion

STS is found to reduce renal crystallization and preserved renal function. Both *in vitro* and *in vivo* studies showed antioxidant potential of STS and it may be acting via scavenging hydrogen peroxide.

References

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Rakesh Bijarnia
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