Pharmacological evaluation of standardized extract of *Zingiber officinale* against arsenic induced nephrotoxicity in laboratory rats

Ms. Pratibha Govind Bhagwat, Dr. P.S.Kute

Dr.Hemant V. Kambale, Ms. Sonali Balasaheb Jadhav

Loknete shri dadapatil pharate college of pharmacy , mandavgan pharata, pune, Maharashtra india.

Corresponding author

Ms. Pratibha Govind Bhagwat

ABSTRACT

The present study investigates the pharmacological potential of a standardized extract of *Zingiber officinale* (ginger) against arsenic-induced nephrotoxicity in laboratory rats. Chronic exposure to arsenic is known to induce oxidative stress, inflammation, and renal dysfunction. In this experiment, rats were divided into control, arsenic-treated, extract-treated, and arsenic + extract-treated groups. Arsenic administration significantly elevated serum creatinine, urea, and uric acid levels, while reducing total protein and antioxidant enzyme activities (SOD, CAT, GSH) in renal tissues. Histopathological examination revealed tubular degeneration and glomerular damage in arsenic-exposed rats. Treatment with the standardized *Z. officinale* extract markedly restored biochemical parameters, enhanced antioxidant defense, and improved renal histoarchitecture. The nephroprotective effect is attributed to the extract's strong antioxidant and anti-inflammatory properties, mainly due to bioactive constituents such as gingerols and shogaols. The findings suggest that standardized *Z. officinale* extract offers significant protection against arsenic-induced renal toxicity and could serve as a potential therapeutic agent for heavy metal–induced nephropathies.

KEY WORDS: Zingiber officinale, arsenic, nephrotoxicity.

INTRODUCTION

The kidneys are a pair of organ that are primarily responsible for filtering metabolites and minerals from the circulatory system. These secretions are passed to the bladder and goes out to the body as the urine. Each kidney is enclosed in a fibrous capsule and is composed of a cortex and an inner medulla. The functional units of kidney called nephrons, with the cortex and medulla filters the blood under pressure then reabsorbs water and selected substances back into blood.

Approximately 1.0 – 1.5 millions nephrons are packed in the average human kidney. The urine thus formed is conducted from the nephrons via the renal tubules into the renal pelvis and from there to ureter, which leads to the bladder. Two basic types of nephrons can be distinguished, **1.** Cortical nephrons - 85% **2**. Juxtamedullary nephrons - 15%.

Renal Diseases (Harper, Guyton A.C. 1996)

Kidneys are the organs that have numerous biological roles. Their primary role is to maintain the homeostat of body fluids by filtering and secreting metabolites (such as urea) and minerals from the blood and excreting the nitrogenous wastes along with water, as urine. Because the kidneys are poised to sense plasma concentrations of ions such as sodium, potassium, hydrogen, and compounds such as amino acids, creatinine, bicarbonate, and glucose, they are important regulators of blood pressure, glucose metabolism, and erythropoiesis.

Renal diseases are classified into the six different physiological categories.

a) Acute Renal Failure (ARF)

A sudden decline in kidney function occurs in patients with pre-existing renal impairment is called Acute Renal Failure. ARF can be diagnostically classified into prerenal, intrarenal and post renal failure.

b) Chronic Renal Failure (CRF)

CRF is due a number of processes leading to permanent loss of kidney function. Its primary causes are high blood pressure and diabetes, but it can also be due to urinary tract obstruction and kidney abnormalities, like polycystic kidney disease.

Causes of CRF includes:

i. Chronic glomerulonephritis

ii. Hypertension

iii. Chronic pyelonephritis

iv. Urinary obstruction

v. Congenital abnormality

vi. Metabolic abnormalitie

c) Hypertensive Kidney Disease

Patients with severe hypertension cause renal lesion that diminish the blood flow or diminished glomerular filtration per nephron.

d) The Nephrotic syndrome

Nephrotic syndrome is a disease, which is characterized especially by loss of large quantities of plasma protein through the urine. Protein loss is due to increased permeability of glomerular membrane.

e) Specific Tubular Disorders

They can cause abnormal reabsorption or lack of reabsorption of certain substances by the tubules. If any required gene is absent or abnormal, then tubules might be deficient of one particular enzyme or carrier. This leads to different tubular disorder.

f) Drug Induced Renal Disease

Drug – induced kidney failure is a major adverse event associated with multiple medication classes. The kidney is particularly vulnerable to drugs and other agents that cause renal damage (nephrolithiasis). Medications as diverse as OTC analgesics (ibuprofen, acetaminophen), antibiotics and chemotherapeutic agents can cause kidney damage.

Acute kidney injury

Acute kidney injury is the new consensus term for acute renal failure. It refers to a clinical syndrome characterized by a fast (hours to days) decrease in renal excretory function, with the accumulation of products of nitrogen metabolism such as creatinine and urea and other clinically unmeasured waste products. Other common clinical and laboratory manifestations include decreased urine output (not always present), accumulation of metabolic acids, and increased potassium and phosphate concentrations. The term acute kidney injury has replaced

acute renal failure to emphasize that a continuum of kidney injury exists that begins long before sufficient loss of excretory kidney function can be measured with standard laboratory tests. The term also suggests a continuum of prognosis, with increasing mortality associated with even small rises in serum creatinine, and additional increases in mortality as creatinine concentration rises (Devarajan, 2006).

The described notions have led to a consensus definition of acute kidney injury by the Acute Dialysis Quality Initiative. This RIFLE (risk, injury, failure, loss, end-stage) criteria (figure 1.1) have been broadly supported with minor modifications by the Acute Kidney Injury Network, and both definitions have now been validated in thousands of patients and seem to work similarly to each other. A new consensus definition merging the RIFLE criteria and the Acute Kidney Injury Network definition has emerged from the Kidney Disease: Improving Global Outcomes (K-DIGO) group (Bellemo *et al*., 2013)

LITERATURE REVIEW

Antioxidant capacity of methanolic extracts from different plant parts of Alpinia galanga. Curcuma longa and Etlingera elatior exhibited varied results. Significant antioxidant activity were found in the polymeric tannin rhizome fraction of A. galanga, non-polymeric phenolic fraction of C. longa rhizomes and its extract, and in the polymeric tannin fraction of E. elatior leaves (Chan et al., 2011).

Mahdavi et al. (2017) found that the Etlingera sayapensis leaf extracts had the strongest antioxidant activity accompanied by the stem and then the extracts from the rhizome. Polarity of the solvent used in solvent extraction specifically influences the antioxidant activity of the extracts, resulting in the maximum antioxidant activity in methanolic extracts while lowest antioxidant activity is found in ethyl acetate extracts respectively.

Studies by Sattar et al. (2013) found that Zingiber officinale and Alpinia allughas had antioxidant activity (percent inhibition) ranging from 26.8 to 68.3 and 14.3 to 58.5 in various solvents, respectively. Generally, the results suggest that both spices are excellent sources of phytochemicals that can be used for medications and/or dietary supplements.

In addition to enhancing scavenging efficiency in Curcuma alismatifolia, Taheri et al (2014) concluded that radiation exposure up to 20 Gy would improve the consistency and volume of bioactive compounds, including phenolic compounds and flavonoids.

Barbosa et al. (2019) found that methanolic extracts from Hornstedtia conoidea leaves had considerably higher antioxidant potential compared with rhizomes. In addition, methanolic extracts from Hornstedtia conoidea leaves have slightly higher phenolic content relative to rhizomes. This is an implication that most of the phenolic compounds in Hornstedtia conoidea are primarily responsible for its strong antioxidant activity.

High phenolic content was found in the freeze dried peels of ginger and turmeric rhizomes. In addition phenolic compunds like 6- gingerol and curcumin increases their antioxidant potential enormously. Therefore, the peels discarded from ginger and turmeric rhizomes may be an fascinating source of bioactive compounds to be introduced as food additives preservatives, beneficial components, nutritional supplements and nutraceuticals because they are not only easy and inexpensive to manufacture but also without any perceptible danger to human safety. During a specific product/service life cycle, food waste including peels may be refined into value-added goods in this manner (Tinello et al., 2019).

MATERIAL AND METHODS

Materials:

Animals:

Sprague Dawley rats weighing 180-200 gm

Instrument used:

Spectrofluorometer

Centrifuge

UV Spectrophotometer

Animal weighing electronic balance

Chemical weighing balance

Tissue Homogenizer

METHODS:

In-vivo parameters:

1)Body weight and urinary output

2)Blood parameter

Ex-vivo parameters:

1)Tissue Parameters

2)Determination of Lipid Peroxidation (MDA content)

3)Determination of Reduced glutathione (GSH)

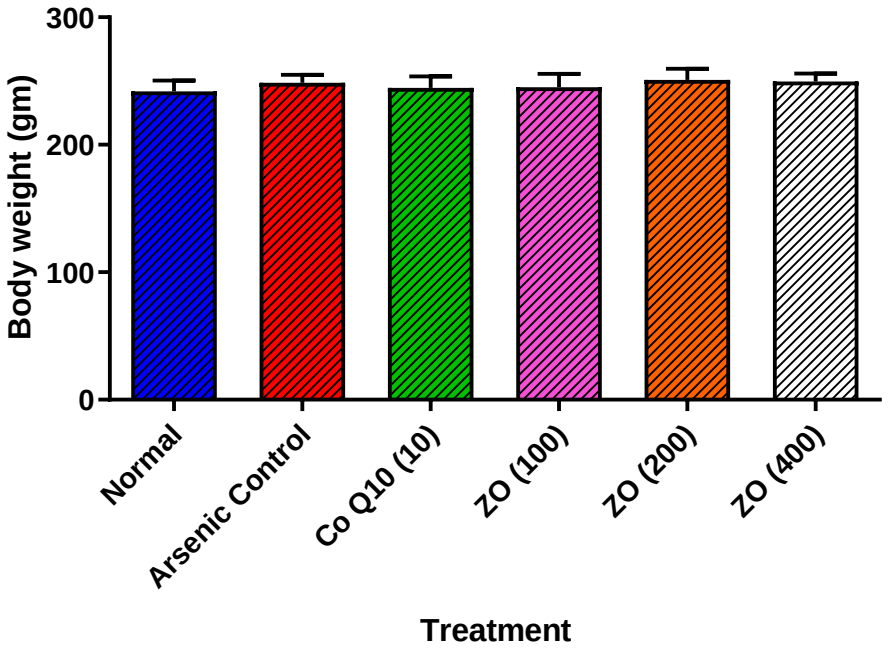
4)Determination of nitric oxide (NO)

5)Determination of tissue protein

RESULTS

1.Effect of *Zingiber officinale* on Arsenic-induced alteration in body weight:

Body weight (gm) - Mean ± SEM					
Normal	**Arsenic Control**	**Coenzyme Q10 (10 mg/kg)**	**ZO (100 mg/kg)**	**ZO (200 mg/kg)**	**ZO (400 mg/kg)**
241.80 ± 3.52	248.50 ± 2.64	244.70 ± 3.64	245.00 ± 4.36	250.80 ± 3.61	249.50 ± 2.60



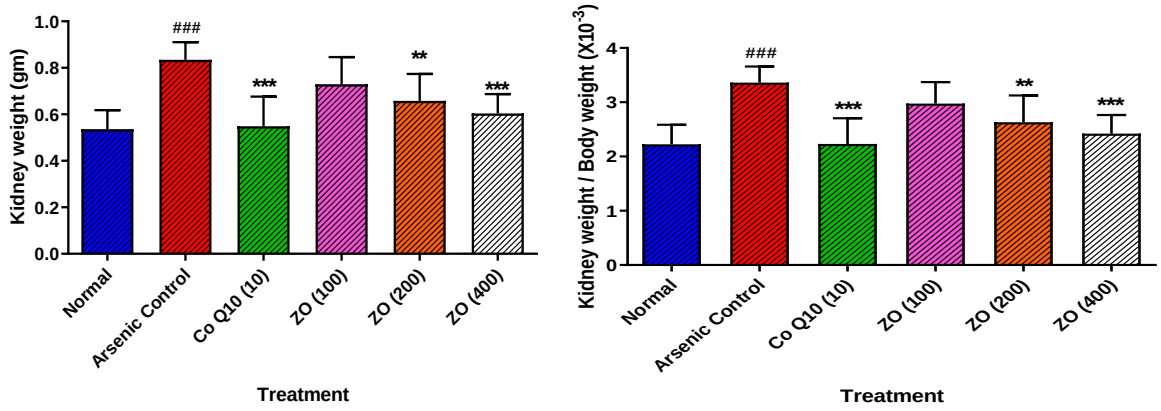
Effect of *Zingiber officinale* on Arsenic-induced alteration in body weight

Data were analyzed by One-Way ANOVA followed by Dunnett's.

When compared to normal group, administration of sodium arsenite did not cause any significant change in body weight in arsenic control group. Treatment of Coenzyme Q10 (10 mg/kg) and *Zingiber officinale* (100, 200 and 400 mg/kg) for 28 days also did not show any significant change in the body weight.

2. Effect of *Zingiber officinale* on Arsenic-induced alteration in absolute and relative kidney weights:

Time (in days)	Absolute kidney weight (gm) and Relative kidney weight - Mean ± SEM					
	Normal	Arsenic Control	Coenzyme Q10 (10 mg/kg)	ZO (100 mg/kg)	ZO (200 mg/kg)	ZO (400 mg/kg)
Kidney weight (gm)	0.54 ± 0.03	0.84 ± 0.03$^{###}$	0.55 ± 0.05***	0.73 ± 0.05	0.66 ± 0.05**	0.60 ± 0.03***
Kidney weight / Body weight ($X10^{-3}$)	2.22 ± 0.15	3.36 ± 0.12$^{###}$	2.23 ± 0.19***	2.97 ± 0.16	2.63 ± 0.20**	2.42 ± 0.14***



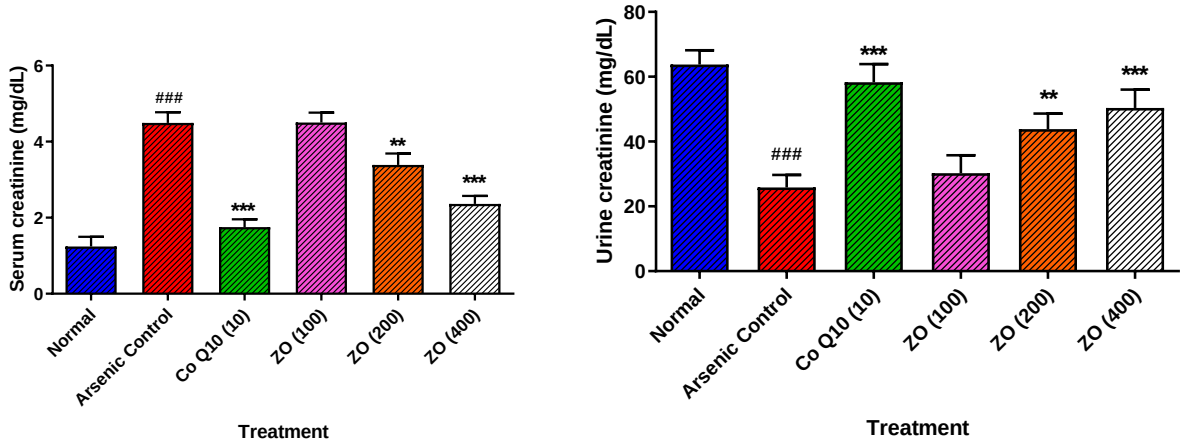
Effect of *Zingiber officinale* on Arsenic-induced alteration in absolute and relative kidney weights

Data were analyzed by One-Way ANOVA followed by Dunnett's. $^{###}P < 0.001$ as compared with normal group and $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with Arsenic Control group on respective days.

When compared to normal group, administration of sodium arsenite cased a significant increase ($P < 0.001$) in absolute and relative kidney weights in arsenic control group. On the other hand, treatment of Coenzyme Q10 (10 mg/kg) resulted in the significant attenuation ($P < 0.001$) of absolute and relative kidney weights as compared with arsenic control group. When compared with arsenic control rats, *Zingiber officinale* (200 and 400 mg/kg) treated rats also showed the significant and dose dependant decreased ($P < 0.01$ and $P < 0.001$) in the absolute and relative kidney weights. Administration of *Zingiber officinale* (100 mg/kg) did not show any significant protection against Arsenic-induced increased renal weights.

3. Effect of *Zingiber officinale* on Arsenic-induced alteration in serum and urine creatinine levels:

Parameter	Serum creatinine (mg/dL) and urine creatinine (mg/dL) - Mean ± SEM					
	Normal	Arsenic Control	Coenzyme Q10 (10 mg/kg)	ZO (100 mg/kg)	ZO (200 mg/kg)	ZO (400 mg/kg)
Serum creatinine (mg/dL)	1.25 ± 0.10	4.49 ± 0.11$^{###}$	1.75 ± 0.08***	4.50 ± 0.11	3.39 ± 0.12**	2.36 ± 0.08***
Urine creatinine (mg/dL)	63.79 ± 1.79	25.84 ± 1.57$^{###}$	58.28 ± 2.29***	30.27 ± 2.25	43.82 ± 1.96**	50.39 ± 2.31***



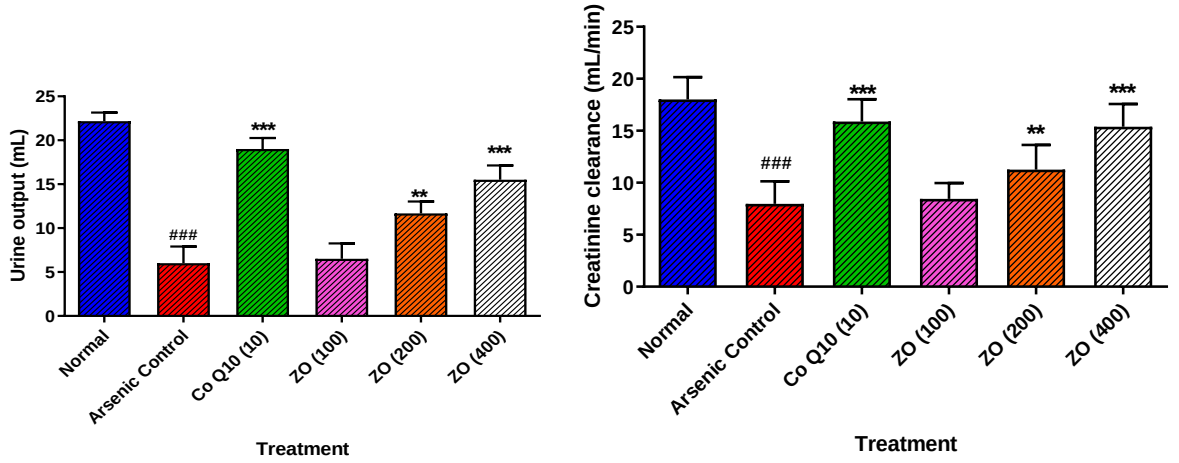
Effect of *Zingiber officinale* on Arsenic-induced alteration in Serum and urine creatinine levels

Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P < 0.001$ as compared with normal group and $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with Arsenic Control group.

On 29th day, the Serum creatinine level was significantly ($P < 0.001$) increased and urine creatinine level significantly ($P < 0.001$) decreased in arsenic control group when compared to normal group. On the other hand, treatment with Coenzyme Q10 (10 mg/kg) showed a significant ($P < 0.001$) decreased and increased in Serum and urine creatinine levels compared to arsenic control group. Treatment with *Zingiber officinale* (200 and 400 mg/kg) showed significant and dose dependant ($P < 0.01$ and $P < 0.001$) decrease in Serum and increase urine creatinine levels compared to arsenic control group.

4. Effect of *Zingiber officinale* on Arsenic-induced alteration in urine output and creatinine clearance level:

Parameter	Urine Output (mL) and Creatinine clearance (mL/min) - Mean ± SEM					
	Normal	Arsenic Control	Coenzyme Q10 (10 mg/kg)	ZO (100 mg/kg)	ZO (200 mg/kg)	ZO (400 mg/kg)
Urine Output (mL)	22.17 ± 0.40	6.00 ± 0.77$^{###}$	19.00 ± 0.52***	6.50 ± 0.72	11.67 ± 0.56**	15.5 ± 0.67***
Creatinine clearance (mL/min)	18.00 ± 0.88	7.96 ± 0.89$^{###}$	15.88 ± 0.88***	8.44 ± 0.63	11.27 ± 0.97**	15.38 ± 0.90***



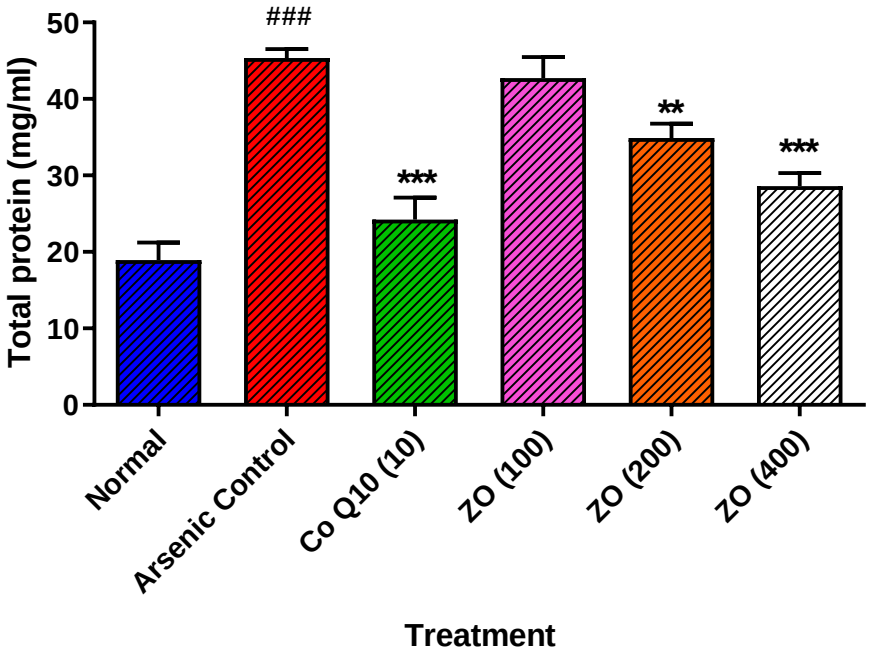
Effect of *Zingiber officinale* on Arsenic-induced alteration in urine output and creatinine clearance level

Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P < 0.001$ as compared with normal group and $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with Arsenic Control group.

Urine output and creatinine clearance level in the arsenic control group was found to be significantly ($P < 0.001$) decrease in normal group. On the other hand, pretreatment of Coenzyme Q10 (10 mg/kg) for 28 days showed significant ($P < 0.001$) increase in urine output and creatinine clearance level as compared to arsenic control group. Treatment with *Zingiber officinale* (200 and 400 mg/kg) also significantly and dose dependently ($P < 0.01$ and $P < 0.001$) increased urine output and creatinine clearance level when compared with arsenic control group.

5.Effect of *Zingiber officinale* on Arsenic-induced alteration in renal total protein level:

Renal total protein (mg/gm) - Mean ± SEM					
Normal	**Arsenic Control**	**Coenzyme Q10 (10 mg/kg)**	**ZO (100 mg/kg)**	**ZO (200 mg/kg)**	**ZO (400 mg/kg)**
18.92 ± 0.95	45.37 ± 0.47$^{###}$	24.21 ± 1.18***	42.73 ± 1.12	34.90 ± 0.77**	28.62 ± 0.70***



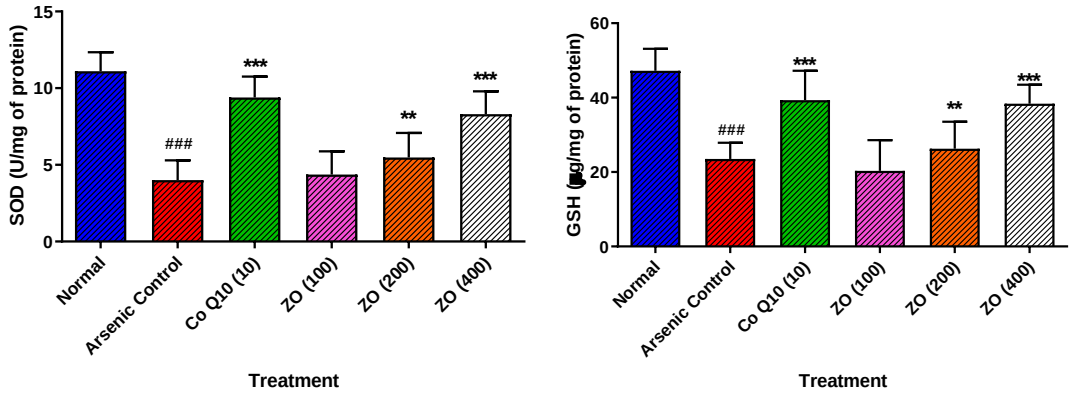
Effect of *Zingiber officinale* on Arsenic-induced alteration in renal total protein level

Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P < 0.001$ as compared with normal group and $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with Arsenic Control group.

There was a significant increase ($P < 0.001$) in renal total protein level in arsenic control group when compared to normal group. Administration of Coenzyme Q10 (10 mg/kg) for 28 days significantly ($P < 0.001$) decrease total protein level in renal tissue compared to arsenic control rats. Treatment with *Zingiber officinale* (200 and 400 mg/kg) also significantly and dose dependently ($P < 0.01$ and $P < 0.001$) decreased the renal total protein level compared to arsenic control rats.

6.Effect of *Zingiber officinale* on Arsenic-induced alteration in renal SOD and GSH level:

Parameter	Renal SOD (U /mg of protein) and GSH µg/mg of protein) levels - Mean ± SEM					
	Normal	Arsenic Control	Coenzyme Q10 (10 mg/kg)	ZO (100 mg/kg)	ZO (200 mg/kg)	ZO (400 mg/kg)
SOD (U /mg of protein)	11.11 ± 0.51	4.01 ± 0.52###	9.40 ± 0.56***	4.37 ± 0.62	5.48 ± 0.65**	8.31 ± 0.61***
GSH (µg/mg of protein)	47.24 ± 2.40	23.51 ± 1.79###	39.32 ± 3.23***	20.32 ± 3.36	26.29 ± 2.96**	38.39 ± 2.08***



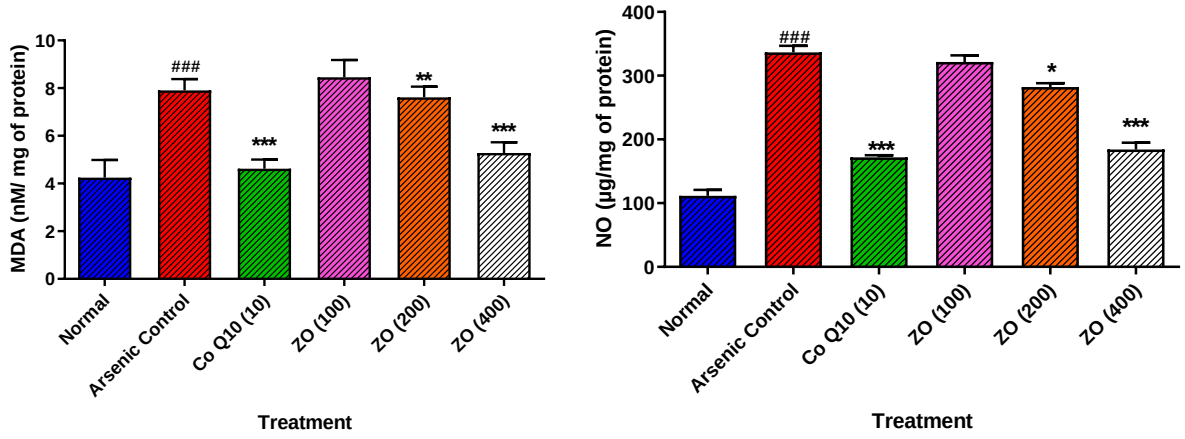
Effect of *Zingiber officinale* on Arsenic-induced alteration in renal SOD and GSH level

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###$P < 0.001$ as compared with normal group and **$P < 0.01$, ***$P < 0.001$ as compared with Arsenic Control group.

The renal SOD and GSH level in the arsenic control rats was significantly decreased ($P < 0.001$) as compared to normal rats. The SOD and GSH level in the renal tissue of Coenzyme Q10 (10 mg/kg) treated rats was significantly increased ($P < 0.001$) as compared to arsenic control rats. The 28 days treatment of *Zingiber officinale* (200 and 400 mg/kg) significantly and dose dependently ($P < 0.01$ and $P < 0.001$) attenuated this Arsenic-induced decreased level of SOD and GSH as compared to arsenic control rats.

7.Effect of *Zingiber officinale* on Arsenic-induced alteration in renal MDA and NO level:

Parameter	Renal MDA (nM/mg of protein), nitric oxide (µg/ml) - Mean ± SEM					
	Normal	Arsenic Control	Coenzyme Q10 (10 mg/kg)	ZO (100 mg/kg)	ZO (200 mg/kg)	ZO (400 mg/kg)
MDA (nM/mg of protein)	4.24 ± 0.30	7.90 ± 0.20###	4.62 ± 0.16***	8.46 ± 0.29	7.62 ± 0.18**	5.28 ± 0.19***
Nitric oxide (µg/ml)	111.40 ± 3.83	336.50 ± 4.22###	171.80 ± 1.41***	321.40 ± 4.25	282.10 ± 2.44*	184 ± 4.37***



Effect of *Zingiber officinale* on Arsenic-induced alteration in renal MDA and NO level

Data were analyzed by One-way ANOVA followed by Dunnett's test. ###$P < 0.001$ as compared with normal group and *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared with Arsenic Control group.

There was significant increase in renal MDA and NO levels in arsenic control rats as compared to normal rats. When compared to arsenic control rats, the MDA and NO level in renal tissue of Coenzyme Q10 (10 mg/kg) was significantly deceased ($P < 0.001$). Administration of *Zingiber officinale* (200 and 400 mg/kg) showed significant and dose dependent ($P < 0.01$ and $P < 0.001$) decreased level of MDA and significant and dose dependent ($P < 0.05$ and $P < 0.001$) decreased level of NO as compared to arsenic control rats.

REFERENCES

1. Bateman, Alex. "The Structure of a Domain Common to Archaebacteria and the Homocystinuria Disease Protein." *Trends in Biochemical Sciences* 22.1 (1997): 12-13.
2. Bellomo, R. (2013). Acute kidney injury markers. *Pathology,45*.doi:10.1097/01.pat.0000426766.98452.6c
3. Birk, J. B., and J. F. P. Wojtaszewski. "Predominant α2/β2/γ3 AMPK Activation during Exercise in Human Skeletal Muscle." *The Journal of Physiology* 577.3 (2006): 1021-032.
4. Bolli, R. (2000). The Late Phase of Preconditioning. *Circulation Research, 87*(11), 972-983. doi:10.1161/01.res.87.11.972
5. Chen, J. (2005). Endothelial Nitric Oxide Synthase Regulates Brain-Derived Neurotrophic Factor Expression and Neurogenesis after Stroke in Mice. *Journal of Neuroscience, 25*(9), 2366-2375. doi:10.1523/jneurosci.5071-04.2005
6. Chertow, G. M. (2005). Acute Kidney Injury, Mortality, Length of Stay, and Costs in Hospitalized Patients. *Journal of*
7. *the American Society of Nephrology, 16*(11), 3365-3370. doi:10.1681/asn.2004090740
8. Cheung, Peter C.f., Ian P. Salt, Stephen P. Davies, D. Grahame Hardie, and David Carling. "Characterization of AMP-activated Protein Kinase γ-subunit Isoforms and Their Role in AMP Binding." *Biochem. J. Biochemical Journal* 346.3 (2000): 659.
9. Cochrane, J., Williams, B. T., Banerjee, A., Harken, A. H., Burke, T. J., Cairns, C. B., & Shapiro, J. I. (1999). Laboratory Study: Ischemic Preconditioning Attenuates Functional, Metabolic, and Morphologic Injury from Ischemic Acute Renal Failure in the Rat. *Renal Failure, 21*(2), 135-145. doi:10.3109/08860229909066978
10. Crute, B. E. "Functional Domains of the Alpha 1 Catalytic Subunit of the AMP-activated Protein Kinase." *Journal of Biological Chemistry* 273.52 (1998): 35347-5354.
11. Culmsee, C., Monnig, J., Kemp, B. E., & Mattson, M. P. (2001). AMP-Activated Protein Kinase is Highly Expressed in Neurons in the Developing Rat Brain and Promotes Neuronal Survival Following Glucose Deprivation. *Journal of Molecular Neuroscience JMN, 17*(1), 45-58. doi:10.1385/jmn:17:1:45

12. Devarajan, P. (2006). Update on Mechanisms of Ischemic Acute Kidney Injury. *Journal of the American Society of Nephrology, 17*(6), 1503-1520. doi:10.1681/asn.2006010017
13. Eltzschig, H. K. (2004). Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: Coordination by extracellular nucleotide metabolism. *Blood, 104*(13), 3986-3992. doi:10.1182/blood-2004-06-2066
14. Fan, L., He, L., Cao, Z., Xiang, J., & Liu, L. (2012). Effect of ischemia preconditioning on renal ischemia/reperfusion injury in rats. *International Braz J Urol Int Braz J Urol, 38*(6), 842-854. doi:10.1590/1677-553820133806842
15. Feng, L., Ke, N., Cheng, F., Guo, Y., Li, S., Li, Q., & Li, Y. (2011). The Protective Mechanism of Ligustrazine against Renal Ischemia/Reperfusion Injury. *Journal of Surgical Research, 166*(2), 298-305. doi:10.1016/j.jss.2009.04.005
16. Gelman, S. (1995). The Pathophysiology of Aortic Cross-clamping and Unclamping. *Anesthesiology, 82*(4), 1026-1057. doi:10.1097/00000542-199504000-00027
17. Grenz, A., Eckle, T., Zhang, H., Huang, D. Y., Wehrmann, M., Kohle, C. Eltzschig, H. K. (2006). Use of a hanging-weight system for isolated renal artery occlusion during ischemic preconditioning in mice. *AJP: Renal Physiology, 292*(1). doi:10.1152/ajprenal.00275.2006
18. Grenz, A., Hong, J. H., Badulak, A., Ridyard, D., Luebbert, T., Kim, J., & Eltzschig, H. K. (2011). Use of a Hanging-weight System for Isolated Renal Artery Occlusion. *Journal of Visualized Experiments JoVE,* (53). doi:10.3791/2549
19. Grenz, A., Osswald, H., Eckle, T., Yang, D., Zhang, H., Tran, Z. V. Eltzschig, H. K. (2008). The Reno-Vascular A2B Adenosine Receptor Protects the Kidney from Ischemia. *PLoS Med PLoS Medicine, 5*(6). doi:10.1371/journal.pmed.0050137
20. Grenz, A., Zhang, H., Eckle, T., Mittelbronn, M., Wehrmann, M., Kohle, C.ltzschig, H. K. (2007). Protective Role of Ecto-5'-Nucleotidase (CD73) in Renal Ischemia. *Journal of the American Society of Nephrology, 18*(3), 833-845. doi:10.1681/asn.2006101141
21. Hallows, K. R., Mount, P. F., Pastor-Soler, N. M., & Power, D. A. (2010). Role of the energy sensor AMP-activated protein kinase in renal physiology and disease. *AJP: Renal Physiology, 298*(5). doi:10.1152/ajprenal.00005.2010
22. Hardie, D. G., Hawley, S. A., & Scott, J. W. (2006). gAMP- activated protein kinase - development of the energy sensor concept. *The Journal of Physiology, 574*(1), 7-15. doi:10.1113/jphysiol.2006.108944

23. Hardie, D. Grahame, Simon A. Hawley, and John W. Scott. "AMP-activated Protein Kinase - Development of the Energy Sensor Concept." *The Journal of Physiology* 574.1 (2006): 7-15.
24. Hausenloy, D. J., & Yellon, D. M. (2009). Preconditioning and post conditioning: Underlying mechanisms and clinical application. *Atherosclerosis, 204*(2), 334-341. doi:10.1016/j.atherosclerosis.2008.10.029
25. Hems, D. A., & Brosnan, J. T. (1970). Effects of ischaemia on content of metabolites in rat liver and kidney in vivo. *Biochem. J. Biochemical Journal, 120*(1), 105-111. doi:10.1042/bj1200105