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ORIGINAL ARTICLE

Effect of Glucocorticoids on Serum Total Protein Level in control and experimental groups induced by Nephrotoxic Poison Concanavalin-A

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ABSTRACT

Background: According to international society of nephrology about 2 million people die with AKI every year. Elder patients and pediatrics have less immunity due to which concurrent administration of medication may damage the nephrons.
Aim: To evaluate the effect of glucocorticoids on serum total protein level in control and experimental groups induced by nephrotoxic poison Concanavalin-A.
Methodology: This experimental study was conducted at Agriculture University of Faisalabad-Pakistan following approval from ethical committee. Subject animals (n=12) were equally divided into 3 groups, 1st was control group, 2nd was treated group and 3rd was untreated group. The collected data was analyzed by using SPSS version 20. ANOVA ware applied with P-value< 0.05
Results: It was found that analysis of serum protein levels showed significant increase in total protein and globulin in Con-A induced toxic group as compare to control group.
Conclusion: It was concluded that dexamethasone treated group showed significant reduction in total protein level and globulin level. Results of serum albumin level were non-significant. Most important of all is serum A/G ratio which showed significant results. Significant reduction in serum A/G ratio in dexa treated group in comparison to untreated group thus dexamethasone was nephro-protective.
Keywords: Glucocorticoids, Serum Protein Markers and Concanavalin-A.

INTRODUCTION

According to international society of nephrology about 2 million people die with AKI every year. Elder patients and pediatrics have less immunity due to which concurrent administration of medication may damage the nephrons[1]. Changes in normal ranges of proteins, metabolic components like urea and creatinine & electrolyte balance indicate the pathological condition of the kidney[2]. Invasion of pathogens stimulates inflammation and tissue damage in body by releasing inflammatory cytokines. Drugs like steroids (Glucocorticoids) plays a role as anti-inflammatory agent and inhibits production of cytokines as well as transforming growth factors[3].

Many endogenous and exogenous factors disturb the physiology of the kidney and reduce its normal functions like detoxification and excretion of waste materials[4]. Literature review showed that ROS plays an important role in inflammation by activating T cells that damage other cells & stimulate inflammation[5]. Previous studies showed that various pathological factors produce ROS due to an imbalance between oxidant and antioxidant defense system[6,7].

Due to renal damage, the levels of albumin & globulin get increased. As a result of foreign body attack, activation of immune system occurs that produce various antibodies (IgA, IgG and IgM) and elevate globulins in blood. However, total proteins and albumin decrease which cause proteinuria[8]. In our daily life chemical exposure like heavy metal, Con-A, carbon tetra chloride from environment cause nephrotoxicity.

Lectin are non-immune originated protein that are present in plants, fungi and animals; have special binding affinity with specifically carbohydrates[9]. Concanavalin A was studies by Jones and johns in 1916. It is lectin (protein) derived from *Canavalia ensiformis*. It specially binds with α-D glucose and α-D mannose also with glycopyranosides[10]. Dexamethasone exhibits anti-inflammatory effect by inhibition of pro-inflammatory mediators including interleukin[11]. Biochemical parameters of kidney as previously described confirm the protective effect of GR ligands.

The objective of the study was to evaluate the effect of glucocorticoids on serum total protein level in control and experimental groups induced by nephrotoxic poison Concanavalin-A**.**

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METHODOLOGY

The study was conducted at Agriculture University of Faisalabad-Pakistan following approval from ethical committee. Subject animals (n=12) were equally divided into 3 groups, 1st was control group, 2nd was treated group and 3rd was untreated group. Total serum protein profile, albumin, globulin and A/G ratio were estimated from blood sample by using serum analyzer.
Chemical and drugs: Concanavalin-A (99% purity) and Dexamethasone were purchased from Sigma Aldrich. Phosphate buffer saline sachets (10X) were purchased from Sigma Aldrich.
Preparation of solutions:
Preparation of Phosphate buffer saline solution: Phosphate buffer saline sachet (10X) was purchased from Sigma Aldrich. Sachet was dissolved in 100 ml of deionized water. The pH of solution was 7.4.
Reconstitution of Concanavalin A: 15 mg of con-A per 5 ml of phosphate buffer saline was dissolved carefully then added 1 drop of 0.1mM Manganese chloride and 1 drop of 0.1mM calcium chloride solution.
Experimental Design: Twelve animals equally divided in 3 groups. All the groups will be kept on routine diet + vehicle (100 µl of ethanol diluted 1:10 in sesame oil) + normal saline for 7 days. Then it was subjected for treatment of con-A separately in each group.
Treatment Protocol:
Day 8th
Group 1: Normal control group=Normal saline and routine diet
Group 2: Untreated group=Vehicle
Group 3: Treated group=Dexamethasone (2mg/kg, i.p)
Day 9th
Group 1: Normal control group=Normal saline and routine diet.
Group 2: Untreated control group
Vehicle + Con-A (15 mg/kg, i.v)
Group 3: Treated group
Dexamethasone (2mg/kg, i.p) + Con-A (15 mg/kg, i.v)

Blood sample was collected at 9th day, centrifuged and stored at -20 centigrade. Biochemical parameters: total protein (g/dl), albumin (g/dl), globulin (g/dl) and A/G ratio was estimated from serum by using calorimetric method.
Statistical analysis: SPSS version 20 was used to analyze the data. ANOVA ware applied. Continuous variables were expressed as Mean±SE. Independent t-Test was used to compare kidney function biomarkers among Con-A treated group and

dexamethasone treated group with P-value<0.05 taken as significant.

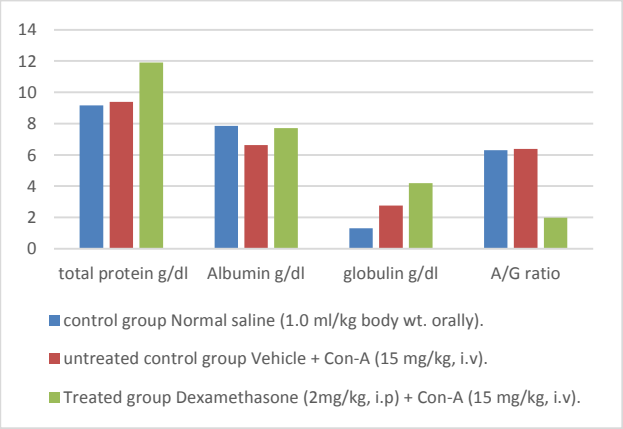
RESULTS

Kidney biomarkers as expected elevated in untreated control group receiving toxicant Con-A 15mg per kg revealed acute renal injury in mice model. Mean± SE of total protein levels were shown in table-1 and figure-1 among different groups.

Table-1: Serum Protein Biomarker Levels at 8th hours among Both Groups

Groups	Treatments	T. protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio
Control group	Normal saline	9.17±0.54	7.85±0.563	1.32±0.174	6.31±1.04
Untreated control group	Vehicle + Con-A.	9.38±1.11	6.62±0.84	2.76±1.22	6.38±3.00
Treated group	Dexamethasone	11.91±0.42	7.71±0.48	4.19±0.52	1.98±0.42

Figure 1: Serum protein biomarker levels (Mean ± SE, g/dL) at 8th hours



DISCUSSION

One of the major health issues all over the world is kidney disease that's increasing day by day. There is significant progress in therapy but the mortality rate is still increasing due to which less than 40% of patients remain alive after 5 years of dialysis. The main cause of kidney problem is nephrotoxicity, toxicant and drugs induce nephrotoxicity by one of the four mechanisms 1) initiation of inflammatory process, 2) membrane disruption, 3) vascular regulation disturbance and 4) production of free radical by oxidative stress.

Nephron is the functional unit of kidney where detoxification and excretion of waste martial and chemical is taken place. Nephrotoxicity is defined as the damaging of nephrons which ultimately disrupt the physiology of the kidney and working of the normal kidney function does not take place. Drug induced nephrotoxicity have different mechanisms like alteration in glomerular filtration, damaging of tubular cells and inflammation. In our daily life chemical exposure like heavy metal, Con-A, carbon tetra chloride from environment cause nephrotoxicity.

Homeostasis of water and mineral ions are regulated by kidneys. Kidney diseases are developed by accumulation of waste materials which disturb the function of renal tubules[13]. It causes nephrotoxicity by activation of T cell and release of inflammatory cytokines at dose of range between 10-30mg/kg[14]. In present study, 15mg/kg dose of concanavalin A was selected to induce nephrotoxicity.

CONCLUSION

It was concluded that dexamethasone treated group showed significant reduction in total protein level and globulin level. Results of serum albumin level were non-significant. Most important of all is serum A/G ratio which showed significant results. Significant reduction in serum A/G ratio in dexa treated group in comparison to untreated group thus dexamethasone was nephro-protective.

Author's contribution: AM&AZ: Conceptualized the study, analyzed the data, and formulated the initial draft, **AJ&AH:** Contributed to the proof reading, **HA&ZI:** Data analysis.

Limitations: Sample size was too small for the study. Resources were limited with financial constrains.

Conflict of interest: Nil

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