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Renal biochemical marker changes in renal protective effects of different doses of Aloe vera on Amphotericin B induced nephrotoxicity in Albino rats: An animal Study

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ABSTRACT

Drug induced nephrotoxicity remains one of the leading causes of acute and chronic kidney diseases in Africa and other developing countries. This can be attributed to environmental pollution due to increased industrialization, poor waste disposal systems, and changes in diets among other factors. The care and treatment of these patients is extremely expensive especially when a patient requires dialysis or kidney transplant. Nephrotoxicity is normally characterized by elevated urea and creatinine levels. The aim of the present study was to evaluate these changes and draw a viable conclusion out of it. In this study a total of 25 rats were used having been calculated using modified resource equation formula. The animals were then systematically sampled and allocated to either pretreatment or treatment groups. Animals in treatment groups were subjected to similar dose of Amphotericin B and different doses of Aloe vera to achieve protection. Later animals were sacrificed humanely and blood collected through cardiac puncture and preserved for renal biochemical analysis. It was noted that there was a significant increase (P=0.0001 and P=0.0001) in both BUN and creatinine respectively in ABG group as compared to pretreatment group. There was a significant reduction (P=0.0001 and P=0.0001) in BUN and creatinine in HDAB group respectively when compared with ABG group. There was no statistical significance (P= 1.000 and P= 1.000) respectively in BUN and creatinine in HDAB group as compared to pretreatment group. It can therefore be concluded that high dose Aloe vera has renal protective benefits in Amphotericin B induced nephrotoxicity as seen in changes of biochemical parameters.

Keywords: Amphotericin B; Aloe vera; Biochemical; Urea; Creatinine; Biomarkers; Histoarchitecture; Oxidative stress; Oxidants; Nephrotoxicity.

1. Introduction

Different methods of detecting acute kidney injury have been developed whereby biochemical parameters such as urea and creatinine have been widely studied (Akaras et al., 2023). Over time, developing countries especially from the African continent have been deeply affected with acute and chronic kidney diseases. The care and treatment of these patients is very expensive especially when the patient requires dialysis and kidney transplant. Evaluation of different renal biochemical parameters is the major outstanding indicator of nephrotoxicity therefore observing such a trend when carrying out this study is key. Very little experimentation has proven no considerable evidence of urea and creatinine increase in patients' serum during renal toxicity (El-Shabrawy et al., 2020). A rise in serum urea and creatinine levels has been believed to be due to nephrotoxicity. This occurs due to altered kidney histoarchitecture that causes structural changes on glomerulus, bowman`s capsule and proximal convoluted tubule. This may cause altered kidney function, accumulation of wastes, blockage of glomerular capillaries and poor circulation of blood. The albino rats have the capacity to reflect the different changes when subjected to any drug because they have a close biological and functional association to human beings. Here albino rats were used based on the following reasons; relatively low costs of maintaining the animals, plentiful and readily available. The animals are small in size, easy to handle and care during the experimental process. They have the ability to withstand a wide range of medicines used in studies (Bailey et al., 2014).

1.1. Study objectives

(i) Assessment of renal biochemical marker changes in renal protective effects of different doses of Aloe vera on Amphotericin B induced nephrotoxicity in Albino rats.

(ii) Evaluation of renal biochemical changes in positive control group (ABG) as compared to pretreatment group.

(iii) Assessment of renal biochemical marker changes in pretreatment group as compared to experimental groups (LDAB, MDAB and HDAB).

(iv) The assessment of renal biochemical marker changes in positive control group (ABG) as compared to experimental groups (LDAB, MDAB and HDAB).

2. Materials and Methods

The study design for this research was posttest only true experimental design whereby an induction is made and the effects compared between control and experimental groups. A total of 25 Albino rats were used for this study whereby these animals were grouped into experimental and control groups. The sample size of 25 Albino rats was calculated using modified resource equation method (Arifin & Zahiruddin, 2017). The animals were assigned to each group by use of systematic simple random sampling. All the rats were subjected to a standard dose of Amphotericin B was given to the positive control group while other groups received similar dose of Amphotericin B concurrently with Aloe vera to achieve protection. The study adapted 50mg/kg of Amphotericin B from (Altuntaş et al., 2014) . Aloe vera dosages were adapted from (Arain et al., 2017) as follows:

High dose 800mg/kg, medium dose 400mg/kg and Low dose 200mg/kg.

2.1. Acquisition of blood samples for renal biochemical analysis

The measurement of all renal biochemical parameters was achieved by filling whole blood drawn by taking a heart puncture. Cardiac puncture is significant in that a lot of blood can be used, for example, one mouse weighing about 150 grams, could provide a large sample (about 10 ml) (Beeton et al., 2007).

2.2. Ethical approval and data analysis

All the ethical requirements were met before the start of this experiment. High hygiene standards were maintained during handling of these animals (Leary et al., 2013). Data was analyzed through SPSS version 26.0. One way ANOVA was used to determine group significance while post hoc Bonferroni was adopted to acquire inter group significance. A P value ≤ 0.05 was adopted and considered significant.

3. Results

3.1. Comparative effects of Aloe vera and amphotericin B on concentration/levels of the biochemical makers

The table below shows a representation of normal values of Blood Urea & Nitrogen and Serum Creatinine levels as adopted from the University of Nairobi Veterinary Laboratory.

Table 1. Normal ranges of biochemical parameter (blood urea & nitrogen and creatinine)

Biochemical parameter	Normal ranges (mmol/L and mg/dl)
Blood Urea and Nitrogen	4.2 – 8.97 mmol/L
Creatinine	0.2 – 0.8 mg/dl

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3.2. Comparative renal biochemical marker findings in control groups

In this segment, a comparison between pretreatment and ABG groups was made using One Way ANOVA to generate an intergroup mean difference and later subjected to post hoc Bonferroni to test for an intergroup significance. It was noted that there was a significant increase (*P=0.0001* and *P=0.0001*) in both BUN and creatinine respectively in ABG group as compared to pretreatment group Table 2.

Table 2. Comparative renal biochemical marker findings between control groups

	Renal biochemical parameters			
Groups	**Blood urea and nitrogen (BUN) in mmol/L**	**P values**	**Creatinine Mg/dl**	**P values**
Pretreatment group (feeds + water)	5.6940±.00894	**0.0001**	0.5660±.03647	**0.0001**
ABG (50mg/kgbwt/day)	9.1900±.09192	**0.0001**	0.9780±.01483	**0.0001**

Key: *All values are expressed as the mean, ± is the standard error of the mean (SEM). The test of significance was performed in rows. Values are expressed as mean ± standard error of mean (n=5), ABG=Amphotericin B group.*

3.3. Comparative mean renal biochemical marker findings among the experimental groups

There was a significant reduction (*P=0.0001* and *P=0.0001*) in BUN and creatinine in HDAB group respectively when compared with ABG group. However, no statistical significance was noted when ABG was compared to LDAB (*P= 1.000* and *P= 1.000*) and MDAB (*P= 0.1920* and *P= 1.000*) respectively in Table 3.

Table 3. Comparative mean renal biochemical marker findings among the experimental groups

Experimental groups	**Mean BUN (mmol/L)**	**Mean creatinine (mg/dl)**
ABG (50mg/kgbwt/day)	9.1900±.09192	0.9780±.01483
LDAB (200mg/kgbwt/day)	9.0680±.18295	0.9720±.13065
P value	**1.000**	**1.000**
MDAB (200mg/kgbwt/day)	9.0080±.14755	0.9240±.03130
P value	**0.1920**	**1.000**
HDAB (200mg/kgbwt/day)	5.5760±.00894	0.5700±.04528
P value	**0.0001**	**0.0001**

Key: *All values are expressed as the mean± the standard error of the mean (SEM). The test of significance was performed in rows. Values are expressed as mean ± standard error of mean (n=5, ABG= Amphotericin B group,*

LDAB= Low dose Aloe vera based treatment, MDAB= Medium dose Aloe vera based treatment, HDAB= High dose Aloe vera based treatment and BUN=Blood urea and nitrogen.

3.4. Comparative mean renal biochemical marker findings between pretreatment and experimental groups

There was no statistical significance (*P= 1.000* and *P= 1.000*) respectively in BUN and creatinine in HDAB group as compared to pretreatment group. On the contrary there was a statistical significance in BUN and creatinine in LDAB (*P=0.0001* and *P=0.0001*) and MDAB (*P=0.0001* and *P=0.0001*) groups when compared to pretreatment group in Table 4.

Table 4. Comparative mean renal biochemical marker findings between pretreatment and experimental groups

Experimental groups	Mean BUN (mmol/L)	Mean creatinine (mg/dl)
Pretreatment group (feeds + water)	5.6940±.00894	0.5660±.03647
LDAB (200mg/kgbwt/day)	9.0680±.18295	0.9720±.13065
P value	**0.0001**	**0.0001**
MDAB (200mg/kgbwt/day)	9.0080±.14755	0.9240±.03130
P value	**0.0001**	**0.0001**
HDAB (200mg/kgbwt/day)	5.5760±.00894	0.5700±.04528
P value	**1.000**	**1.000**

Key: *All values are expressed as the mean± the standard error of the mean (SEM). The test of significance was performed in rows. Values are expressed as mean ± standard error of mean (n=5, ABG= Amphotericin B group, LDAB= Low dose Aloe vera based treatment, MDAB= Medium dose Aloe vera based treatment, HDAB= High dose Aloe vera based treatment and BUN=Blood urea and nitrogen.*

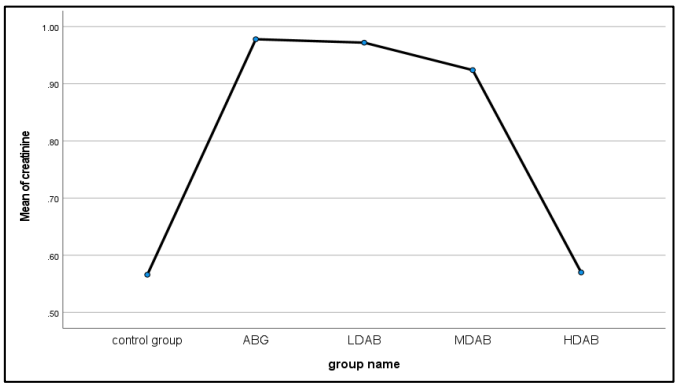

Figure 1. Comparative mean creatinine in control and experimental groups

Key: *ABG= Amphotericin B group, LDAB= Low dose Aloe vera based treatment, MDAB= Medium dose Aloe vera based treatment and HDAB= High dose Aloe vera based treatment.*

It can be seen that the mean levels of creatinine increased markedly in ABG group then dropped steadily in LDAB, MDAB and HDAB whereby HDAB had a significant drop level.

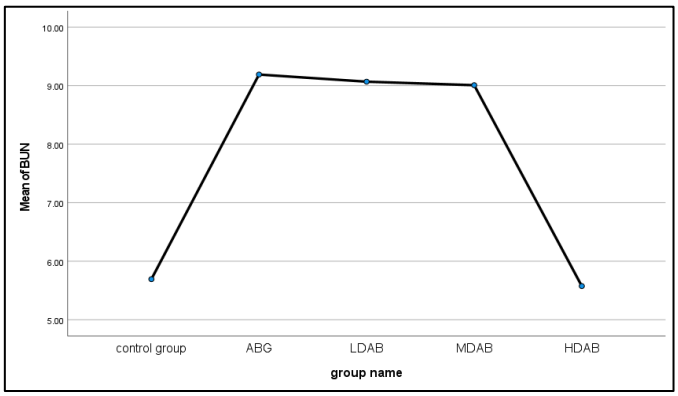

Figure 2. Comparative mean BUN in control and experimental groups

Key: *ABG= Amphotericin B group, LDAB= Low dose Aloe vera based treatment, MDAB= Medium dose Aloe vera based treatment, HDAB= High dose Aloe vera based treatment and BUN=Blood urea & nitrogen.*

In figure 2 above, it can be deduced that the mean level of BUN significantly increased in ABG group and the vice versa can be equally reported in HDAB group.

4. Discussion

Urea is a chemical substance produced when proteins and ammonia is broken in the liver. On the other hand, creatinine is the end product formed as a result of normal muscle activities or in event of muscle breakdown of body. Urea and creatinine are commonly used in developing countries to detect kidney malfunction and estimate resultant type of kidney injury. The main reason as to why these biomarkers are easily utilized is because they are filtered by glomerulus and released into urine where they are single handedly expelled as nitrogenous wastes. Therefore, it is prudent to note that any injury to the glomerulus or kidney tubules can likely lead to impaired kidney functions.

In the present study, the use of Amphotericin B which is a common nephrotoxic agent compromised glomerulus and proximal convoluted tubule leading to distortion of cell membrane, histoarchitectural structural changes thus reducing filtration rate of kidney. Once this damage has taken place there will be accumulation of nitrogenous wastes that complicates to reduced kidney perfusion. This is evident in this study where there was a significant increase in BUN and creatinine levels in ABG, LDAB and MDAB groups as compared to pretreatment groups which had levels within normal ranges. Similar denotation was observed by (Liu et al., 2018) when assessing rat renal biochemical changes.

The above similarity is due to increased production of oxidative stress and inflammatory markers that are known to cause damage to kidney tubules and glomerulus. Once these structures are damaged, there will be a reduction in glomerular filtration rate thus leading to increase in serum urea and nitrogen. Contrary to this, (Wu et al., 2017) recorded a reduced level of urea. It is worth noting that the agent used by the mentioned researcher was metabolized by both liver and kidney which likely affects its levels.

There was a significant reduction in levels of urea in HDAB group as compared to ABG group. These findings concur with (Bannoth, 2022) where by rats that were exposed to '*Amnosa squmosa*' extract recorded a remarkable elevation in serum urea levels signifying protection. Similar findings were reported by (Ali et al., 2005; Hassan et al., 2019) when assessing protective mechanisms of curcumin. It is worth to note that curcumin has been referred to as it exhibits a similar nephroprotection pathway as Aloe vera where they majorly target glomerulus. The significant reduction noted is due to antioxidative features exhibited by Aloe vera, which prevents inflammation and scavenge for radical oxidants produced during Amphotericin B nephrotoxicity. This in turn improves renal blood circulation and excretion patterns of kidney.

The rise in levels of creatinine in ABG group was possible due to renal damage and accumulation of injured epithelial cells within glomerular vessels. This was similarly reported by (Kundu et al., 2012) in Bangladesh. A significant reduction in levels of creatinine in HDAB group was observed when compared to ABG group. This reduction in creatinine levels paints a picture of positive nephroprotection exhibited by Aloe vera on Amphotericin B induced toxicity.

Normally during drug induced nephrotoxicity, a lot of reactive oxygen radicals and nitrogen oxide are produced. These substances are known to damage kidney tubules alongside glomerulus which alters the histo-architecture of kidney structures. This therefore, reduces kidney`s ability to excrete toxic wastes. However, good news is that utilization of Aloe vera (HDAB) group causes a counter-reaction to this pathway by producing anti-inflammatory and antioxidant components. This steers up blood supply to glomerular vessels, waste and drug clearance. This concurs with (Baradaran et al., 2015; Ribeiro et al., 2023) in their research quest to evaluate effect of antioxidant use in chronic kidney diseases. This segment therefore, rejects null hypothesis and upholds that in deed, Aloe vera has a significant effect on the levels of renal biochemical parameters.

5. Conclusion

It can be concluded that high dose Aloe vera has protective benefits in nephrotoxicity caused by Amphotericin B as witnessed in this study. Therefore, further studies can be done on: (i) Utilization of advanced renal biochemical markers like KIM-1 and KIM-2 to assess the renal protective effects of Aloe vera in Amphotericin B induced nephrotoxicity, and (ii) Histoimmunochemistry can be conducted to assess the sensitivity and type of receptors involved in renal protection of Aloe vera in Amphotericin B induced nephrotoxicity.

Declarations

Source of Funding

This study did not benefit from grants from any non-profit, public or commercial funding agency.

Competing Interests Statement

The authors have declared that no competing financial, professional or personal interests exist.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

Both the authors took part in literature review, analysis, and manuscript writing equally.

Ethical Approval

All the ethical requirements were met before the start of this experiment.

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