Article

Effects of Ashwagandha (Withania somnifera) Root Extract Against Gentamicin Induced Changes of Serum Electrolytes in Rats

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Abstract

Background: Regulation of electrolytes and body fluids are essential for maintaining the body homeostasis. Kidney plays an important role for these regulations. Higher doses of drugs, toxins, infectious agents, chemicals etc. can causes kidney damage and ultimately electrolytes disturbances can be occurred. Ashwagandha (Withania somnifera) is an herbal plant may have some role on serum electrolytes balance. **Objective:** To observe the effects of ashwagandha (Withania somnifera) root on serum electrolytes against gentamicin induced nephrotoxicity in Wistar albino rats. **Methods:** This experimental study was carried out in the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka from 1st July 2010 to 30th June 2011. A total number of 35 Wistar albino rats, age from 90 to 120 days, weighing between 150 to 200 grams were selected for the study. After acclimatization for 14 days, they were divided into control group and experimental group. Control group was again subdivided into baseline control, (10 rats) and gentamicin treated control group, (10 rats). Again, experimental group (gentamicin treated group after ashwagandha treatment) consisted of 15 rats. All groups of animals received basal diet for 22 consecutive days. In addition to this, gentamicin treated control group also received gentamicin subcutaneously (100mg /kg body weight/day) for the last eight (15th to 22nd day) consecutive days. Again, gentamicin treated group after ashwagandha treatment received ashwagandha root extract (500mg/kg body weight/day, orally) for 22 consecutive days and gentamicin subcutaneously (100mg/kg body weight /day) for last eight (15th to 22nd day) days. All the animals were sacrificed on 23rd day. Then blood samples were collected and kidney weight was measured. For assessment of kidney function, some serum electrolyte levels e,g. serum sodium, potassium and chloride ion levels were estimated by ion selective electrode (ISE) electrolyte auto analyzer method, by using Biolyte 2000 auto analyzer . However, body weight and kidney weight of the animals were measured to assess the nephrotoxicity in these groups of animals. All these tests were done in the laboratory of Department of Physiology and Biochemistry, SSMC. Statistical analysis was done by one way ANOVA and Bonferroni tests as applicable. **Results:** The serum sodium and chloride ion levels were almost similar in all the groups and the differences were not statistically significant. The mean serum levels of potassium ion were significantly ($p<0.001$) lower in gentamicin treated group and ($p<0.05$) in gentamicin treated group after ashwagandha treatment in comparison to that of baseline control group. But this level of gentamicin treated group after ashwagandha treatment was significantly ($p<0.01$) higher than that of gentamicin treated group. Initial body weight was almost similar and no significant difference of this value was observed among the groups. Whereas, the final body weight was significantly ($p<0.001$) lower in gentamicin treated control group and in gentamicin treated group after ashwagandha treatment than that of baseline control group. Again this level of gentamicin treated group after ashwagandha treatment was significantly ($p<0.05$) higher in comparison to that of gentamicin treated control group. The kidney weight was significantly ($p<0.01$) higher in gentamicin treated control group when compared to that of baseline control and gentamicin treated group after ashwagandha treatment. Whereas, kidney weight of gentamicin treated group after ashwagandha treatment and of baseline control group was almost similar and showed no statistically significant difference of this value between this two groups. **Conclusion:** Ashwagandha (Withania somnifera) root extract may have some role in maintaining some of the serum electrolyte levels within normal limit, which indicates its nephroprotective effects against gentamicin induced toxicity.

Key words: Electrolytes, Ashwagandha, Gentamicin

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Introduction

Acids, bases and salts are collectively called electrolytes[1]. Electrolytes are involved in many metabolic and homeostatic functions, of our body[2]. Abnormal electrolytes concentrations reflect altered metabolic status[3]. Electrolyte imbalance can leads to serious consequences as it affects the homeostasis of the body. Commonly measured electrolytes are good indicators of kidneys function[1].The kidney is an essential organ plays an important role in regulation of electrolytes and body fluids[4]. The test of electrolytes includes the measurement of sodium, chloride, potassium, bicarbonate ions for both diagnosis and management of renal, endocrine, acid-base, water balance and many other condition[5]. Altered levels of these electrolytes are commonly occurs in renal dysfunction and may be life threatening[6].

The amino glycoside antibiotic gentamicin is widely used in the treatment of infection caused by gram negative bacteria[7]. Drug metabolite may have higher activity and greater toxicity than the original drug. Metabolites of the drugs that are excreted from kidney may also cause cellular damage leading to kidney dysfunction[8]. Gentamicin is a well recognized nephrotoxic drugs produce renal tubular necrosis[9]. A less well documented effect of gentamicin is a disturbance of electrolyte homeostasis.[10] Gentamicin causes electrolytes disturbance particularly serum potassium, sodium, magnesium, calcium etc[11].

Ashwagandha (Withania somnifera,) belongs to the family of Solanaceae, is the traditional Ayurvedic medicine[12]. The roots of ashwagandha contains withaferin A and withanolides, the active ingredients contribute to the most of the biological actions[13]. Roots of this plant may have some therapeutic effects[12]. Till today, no side effects have been found in ashwagandha[14].

Again, different researchers from different countries have been studied the effect of ashwagandha (Withania somnifera) on electrolytes. Some researchers observed that ashwagandha significantly decreases the serum electrolytes[15] which may be due to its diuretic effect. Moreover, other investigators found that root of this plant causes non significant change of serum sodium ion but significantly increase serum potassium ion[16].

Renal failure with electrolyte disturbance is potentially serious and causes high morbidity and mortality in our country. In recent years, great effort has been focused on traditional and herbal medicine for the treatment of renal disease along with electrolyte balance. But little is known on this aspect in Bangladesh. Therefore, the present study was carried out to observe the effects of ashwagandha (Withania somnifera) root extract on serum electrolyte levels in gentamicin intoxicated rats. It is also expected that the result of this study would make the ashwagandha acceptable among the people for maintaining the electrolyte concentration in the body.

Methods

This experimental study was conducted between July 2010 to June 2011 in the Department of Physiology, SSMC, Mitford, Dhaka. A total number of 35 apparently healthy Wistar albino male rats, weighing between 150 to 200 grams, age from 90 to 120 days were used. The animals were purchased from animal house of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Ethical permission was taken from the Institutional Ethics Committee (IEC) of SSMC, Dhaka. Prior conducting the study, the animals were acclimatized for 14 days at 23 ± 2^0C room temperature under 12 hour dark- light cycle. During this period, they had free access to food and water ad libitum. After 14 days of acclimatization, body weights were measured (initial bw), again, final body weights (final bw) of rats before anaesthetized on 23rd day were taken. The rats were divided into control group and experimental group. Control group was again subdivided into baseline control, consisted of 10 rats) and gentamicin treated control group (consisted of 10 rats). Again, experimental group

(gentamicin treated group after ashwagandha treatment) consisted of 15 rats. All groups of animals received basal diet for 22 consecutive days. In addition to this, gentamicin treated control group also received gentamicin subcutaneously (100mg /kg body weight/day) for the last eight (15^{th} to 22^{nd} day) consecutive days. Again, gentamicin treated group after ashwagandha treatment received ashwagandha root extract (500mg/kg body weight/day, orally) for 22 consecutive days and gentamicin subcutaneously (100mg/kg body weight /day) for last eight (15^{th} to 22^{nd} day) days. After giving gentamicin and ashwagandha all the animals including baseline control rats, were anaesthetized with the help of chloroform and sacrificed on 23^{rd} day. Their blood samples were collected and kidney was removed and was washed in ice cold saline. Then it was wiped in tissue paper, weighed by electric balance analyzer. Blood was centrifuged at the rate of 4000 rpm for 5 minutes and supernatant serum was collected. Out of 35 serum samples, four (4) were hemolyzed. So, thirty one (31) samples were estimated. For assessment of kidney function, some serum electrolyte levels e,g. serum sodium, potassium and chloride ion levels were estimated by ion selective electrode (ISE) electrolyte auto analyzer method, by using Biolyte 2000 auto analyzer[17] . However, body weight and kidney weight of the animals were measured to assess the nephrotoxicity in these groups of animals. All these tests were done in the laboratory of Department of Physiology and Biochemistry, SSMC. Statistical analysis were done by one way ANOVA and Bonferroni test as applicable by using SPSS windows, version 16.

Preparation of root extract of ashwagandha (Withania somnifera):

Ashwagandha is cultivated and harvested in the Ayurvedic nursery of Hamdard Laboratories, Meghna. After collecting the roots of this plant, it was dried in sunlight for 2 days, crushed in an electrical grinder into powder. Then the powder was extracted in methanol, filtered, evaporated by rotatory evaporator and dried. After that, the dried root extract of ashwagandha was dissolved by propylene glycol (2ml/kg body weight) and finally mixed with distilled water for feeding.

Results

Body weight and kidney weight are shown in (Table I).

Table I : Body weight and kidney weight in different groups (n=31)

Groups	Body weight (g)		Kidney
	Initial (Day 1)	Final (Day 23)	weight (g)
A_1(n=9)	181.13 ± 2.64	187.18 ± 3.66	0.57 ± 0.03
	(177.12-185.24)	(183.10-194.09)	(0.54-0.61)
A_2(n=9)	182.30 ± 2.80	176.83 ± 3.69	1.20 ± 0.14
	(178.04-185.92)	(169.67-180.28)	(1.07-1.38)
B(n=19)	181.85 ± 3.13	180.46 ± 2.59	0.58 ± 0.01
	(177.10-186.92)	(175.92-184.26)	(0.55-0.59
Statistical analysis			
		P value	
A_1 vs A_2	0.189^{ns}	0.000^{***}	0.003^{**}
A_1 vs B	0.285^{ns}	0.000^{***}	0.188^{ns}
A_2 vs B	0.363^{ns}	0.012^{*}	0.007^{**}

Results are expressed as Mean ± SD. Statistical analysis was done by ANOVA test and then Bonferroni test was performed to compare between groups. Figures in parentheses indicate maximum& minimum values.
Group A_1=Baseline control group Group A_2=Gentamicin treated control group
Group B= gentamicin treated group after ashwagandha treatment
n: total number of animal ns: non-significant * : $p < 0.05$
** : < 0.01 ***: $p < 0.001$

Initial body weights of all the animals were almost similar and showed no significant difference of this value among the groups. Whereas, the final body weight was significantly ($p<0.001$) lower in gentamicin treated control group and in gentamicin treated group after ashwagandha treatment than that of baseline control group. Again this level of gentamicin treated group after ashwagandha treatment was significantly ($p<0.05$) higher in comparison to that of gentamicin treated control group.

The kidney weight was significantly ($p<0.01$) higher in gentamicin treated control group when compared to that of baseline control and gentamicin treated group after ashwagandha treatment. Whereas, kidney weight of gentamicin treated group after ashwagandha treatment and of baseline control group was almost similar and showed no significant difference of this value between this two groups.

The mean serum sodium and chloride ion levels were slightly higher in gentamicin treated control group and gentamicin treated group after ashwagandha treatment in comparison to those of baseline control group. Whereas, these levels were slightly lower in gentamicin treated group after ashwagandha treatment than those of gentamicin treated control group. Although, these differences were not statistically significant (Figure 1). Again, the mean serum potassium ion level was significantly lower in gentamicin treated control group ($p<0.001$) and gentamicin treated group after ashwagandha treatment ($p<0.05$) in comparison to that of baseline control group. Again, this level was significantly ($p<0.01$) higher in gentamicin treated group after ashwagandha treatment than that of gentamicin treated control group. (Figure 2).

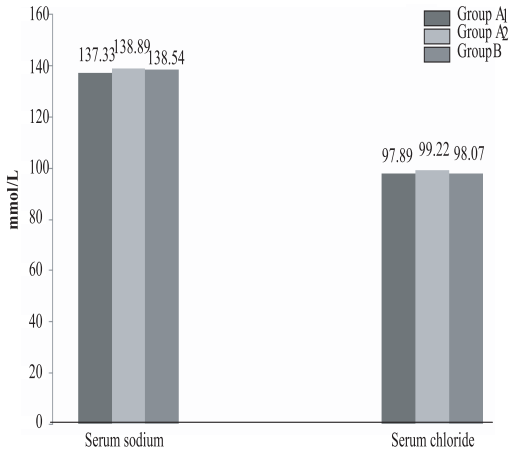

Figure 1: Serum sodium and chloride ion levels in different groups of rats (n=31). Group A_1: Baseline control group Group A_2 : Gentamicin treated control, Group B: Gentamicin treated group after ashwagandha treatment

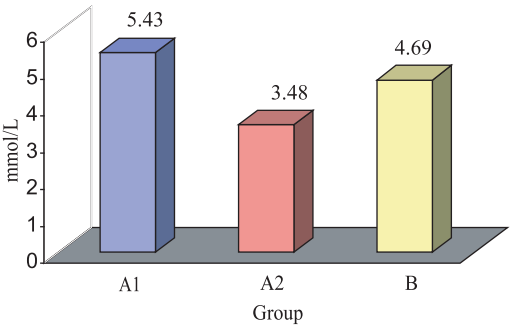

Figure 2: Serum potassium ion level in different groups of rats (n=31). Group A_1:Baseline control group, Group A_2: Gentamicin treated control group, Group B (Gentamicin treated group after ashwagandha treatment)

Discussion

In this study lower body weight was found both in gentamicin treated control rats and also in gentamicin treated group after ashwagandha treatment. Again, higher kidney weight was found in gentamicin treated control rats, whereas kidney weight was almost similar in gentamicin treated

group after ashwagandha treatment and baseline control group. This finding is in consistent with that of some other investigators[12].

In the present study, non-significant changes of serum sodium and chloride ion levels were observed among the groups. This finding is in agreement with that of some other researchers[3,16,18]. Again, some other researchers observed significantly lower level of serum sodium ion in gentamicin treated group after ashwagandha treatment in comparison to that of baseline control group[15] . The inconsistent of the result may be due to that ashwagandha was used for 30 days to potentiate diuretic effect in that study in contrast to 22 days administration of ashwagandha in the animals of present study.

Moreover, in this study, hypokalemia was observed in gentamicin treated control group which was improved in gentamicin treated group after ashwagandha treatment towards baseline control. Similar finding was also reported by some other researchers[9,16,18,19].

It has been suggested that, toxic dose of gentamicin may decrease the plasma magnesium concentration. This hypomagnesaemia may reduce the body weight[20]. Again, some other investigators suggested that gentamicin toxicity causes development of uremia, which in turn causes loss of appetite and decrease in body weight[16]. Moreover, gentamicin is injurious to renal epithelial cells, thus may decrease water reabsorption and ultimately decrease the body weight[21].

Again, Gentamicin decreases serum potassium ion level, may be due its stimulatory effect on aldosterone secretion[22]. Furthermore, glomerular dysfunction and altered tubular reabsorption causes slightly increased level of sodium and decreased level of potassium and magnesium in gentamicin treated rats[18, 23].

However, some researchers suggested that, presence of active ingredients like withanolides, may be responsible for diuretic activity of ashwagandha by inhibiting the sodium ion reabsorption in kidney tubules [24].

Again, some other researchers suggested that, ashwagandha root contained sitoindosides VII-X and withaferin A, have antioxidant activity by enhancing the free radical scavenging enzymes such as, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)[12]. Therefore, causes improvement of body weight[16]. Again, anti-inflammatory action of its active ingredients may be helpful in decreasing kidney weight[16].

In the present study, nephrotoxicity was observed in rats treated with gentamicin as evidenced by some of the electrolyte imbalance to some extent. This is further supported by an increase in kidney weight in nephrotoxic rats of present study which may be due to increased production of free radicals, which initiate lipid peroxidation and subsequent cellular damage. Significant decrease final body weight of this group of animals is also in favor of this finding.

Again, in this study slightly lower level of serum sodium and chloride ions along with significantly higher level of potassium ion and lower in kidney weight, that comes to almost normal level in the gentamicin treated group after ashwagandha treatment provides an evidence that, the root extract of this ashwagandha plant may have nephroprotective effect against gentamicin toxicity. These effects are most likely due to presence of some active ingredients in ashwagandha root which have antioxidant property. However, the exact mechanism involved cannot be found out from this type of study due to time and financial constrain.

Conclusion

From this study, it can be concluded that ashwagandha (Withania somnifera) root extract can maintain some of the serum electrolyte levels to some extent, e,g. serum sodium, potassium and chloride ion levels in gentamicin intoxicated rats, may be due to its antioxidant property. This

effect of ashwagandha root reflects its nephroprotective effects against gentamicin toxicity.

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