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PHARMACEUTICAL SCIENCE
Research Article

Toxicity Effects of Both *Alum* and *PolyDADMAC* on Newzealand Rabbits

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Received: March 16, 2015; **Published:** June 05, 2015

Abstract

Six heads of New Zealand rabbits were divided into 2 groups. Group 1 animals were the undosed controls. The test group was given 1% alum and 1% *polyDADMAC* at 1:2 for a period of 10 weeks after an adaptation period of two weeks during which the animals were under ideal experimental conditions.

Clinical signs were closely observed with postmortem and histopathological examinations. Chemical investigations included enzymatic concentrations of ALP, GOT, CK, GPT and LDH and metabolic changes of albumin, urea, total protein, cholesterol, bilirubin, glucose and creatinine. Fluctuations in electrolyte levels of Mg, Fe, Na, K, Ca and P were monitored together with hematological changes in Hb, PCV, RBCs and WBCs.

Rabbits of group 2 were off-food, showed salivation, vomiting and diarrhea accompanied by hind limb paralysis. The mortality rate was 100 percent. Their lungs showed adhesions, congestions and haemorrhages. Intestinal and stomach flatulence were also obvious. Cardiac, renal and liver congestions were sometimes accompanied by haemorrhages. The livers and kidneys showed focal areas of necrosis. On histopathological assessment, the livers of rabbits of group 2 showed necrosis, haemorrhages and lymphocyte infiltrations, where as those of the control (group 1) were quite normal. Variable ($P < 0.05 - 0.01$) increases in the serum activities of ALP, GOT, GPT and LDH were observed. Significant ($P < 0.01 - 0.001$) decreases were observed when testing their serum level of CK compared to the control group, The serum of test group revealed decreasing ($P < 0.05$) albumin and increasing ($P < 0.05$-0.001) cholesterol and bilirubin concentrations, while serum levels of total protein, glucose, urea and creatinine were not different ($P > 0.05$) from those of the un-dosed group 1 rabbits. The concentrations of all the electrolytes in the serum of test rabbits showed significant ($P < 0.01 - 0.001$) decreases, when compared to the control group.

Values of the PCV of rabbits in groups 2 were decreased ($P < 0.01$) compared to the control rabbits. The RBCS and WBCs values of rabbits in groups 2s were increased ($P < 0.01$) in compared to the un-dosed control rabbits. Normal haematological values were recorded in the control group.

On evaluation of the above results, the interactive water treatment with 1% alum and 1% *polyDADMAC* at 1:2 was considered toxic to Newzealand rabbits at the dose rates tried .Practical implications of the results were highlighted and suggestions for future work were put forward.

Keywords: *Alum and PolyDADMAC; Drinking Water; Interactive Toxicity; Newzealand Rabbits*

Introduction

In the Sudan, the first use of a chemical to reduce the turbidity of the Nile water especially during the flood season to be suitable for the healthy human consumption. The disposal system to eliminate the outcome of the sludge of the chemical reactions that happen during treatment of the turbid water is not adjusted well to the laws of environmental health regulations. Through-out the time Alum did not give satisfactory results in the reduction of turbidity of water, so new methodologies were introduced with special preference in use of *polyDADMAC* polymer. Toxicological were always lacking and hence subjected this use to a lot of debate. This experiment is a trial to lead the way out.

Materials

Animals

Six, 5-7 month old mixed, clinically healthy Newzealand rabbits were purchased from Balsam Pharmaceutical Laboratories in the vicinity of Khartoum North, housed and were given prophylactic doses of oxytetracycline 20%. The rabbits were ear tagged, given a two-week preliminary period during which Lucerne was fed and Nile drinking water was provided ad libitum.

Administration of the doses

Test materials of polyDADMAC and alum (AlSO4) were prepared in separate stocks each as a 1% solution of each. The two test solutions were blended at the dose rate of 1:2 of the polymer and alum respectively. Animals were weighed and distributed into two experimental groups. Test blend was given orally to rabbits of group 2 in drinking water obtained from the Nile daily. The untreated controls were the rabbits of group 1.

Methods

Clinical signs: were observed and postmortem changes were recorded.

Histological methods: The specimens were collected immediately after death or slaughter and fixed in 10% formal saline, embedded in paraffin wax, sectioned at 5 µm and stained with haemotoxylin and eosin (H & E) using Mayer's haemalum.

Haematological methods: These were described by Schalm [1] 1965. Blood samples from rabbits were collected into clean dry bottles containing the anti-coagulant heparin from the ear vein. Hb concentrations together with counts of RBCs, WBCs and PCV were then investigated.

Chemical methods: Blood samples obtained the ear vein of rabbits before and after dosing with the joint solution. Venous blood samples were centrifuged at 3000 rpm for 5 minutes and stored at -20°C until analyzed for enzymatic activities of ALP, GOT, CK, GPT and LDH; The metabolic changes in response to treatment in albumin, urea, total protein, cholesterol, bilirubin, glucose and creatinine and finally the changes in the electrolytes magnesium, iron, sodium, potassium, calcium and phosphorus.

Statistical methods: The difference between mean values of data was analyzed by the un-paired students- t-test [2].

Results

Clinical signs: Rabbits of group 2 were off-food, showed salivation, vomiting and diarrhea. They moved slowly and finally they showed hind limb paralysis. The mortality rate was 100 percent. Normal behavior was observed in rabbits of group 1.

Postmortum changes: The lungs of the rabbits of group 2 showed adhesions, congestions and haemorrhages. Intestinal and stomach flatulence were also obvious. Cardiac, renal and liver congestions were sometimes accompanied by haemorrhages. The livers and kidneys showed focal areas of necrosis. Rabbits of group 1 showed normal organs.

Histopathology: In the livers of rabbits of group 2 necrosis, haemorrhages and lymphocyte infiltrations were clearly seen.

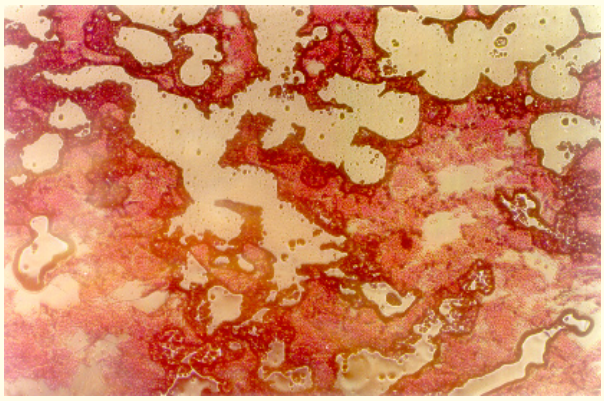

Figure 1: *Lung emphysema in a rabbits of group 2 dosed with combined 1% alum and 1% polymer at 1:2 in drinking water. X100*

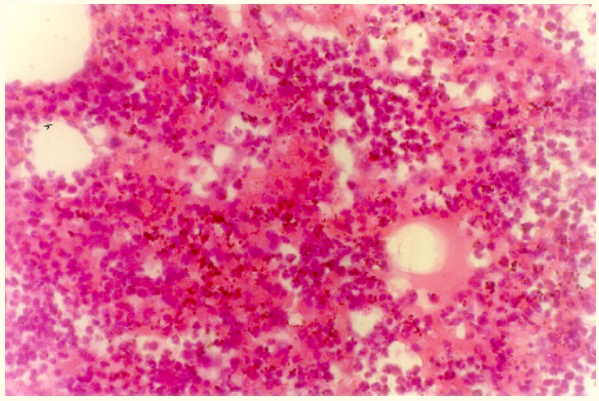

Figure 2: *Haemosiderin deposition in the spleen of a rabbits of group 2 dosed with combined 1% alum And 1% polymer in drinking water. X100*

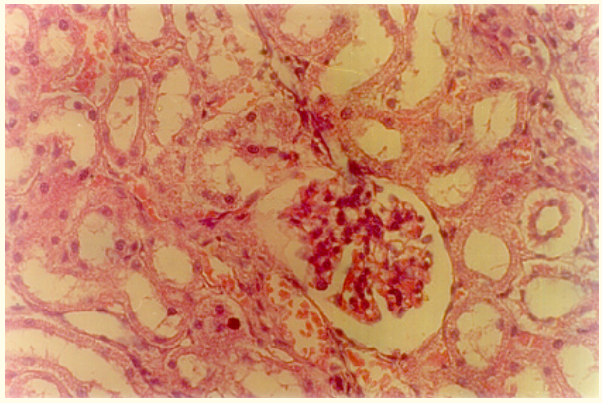

Figure 3: *Necrosis in the glomeruli and hemorrhage in the tubules of a rabbits dosed with combined 1% alum and 1% polymer at 1:2 in drinking water. X100.*

Normal tissues were seen in the control rabbits.

Chemical enzymatic changes

Group/Dose	ALP (iu/l)	GOT (iu/l)	CK (iu/l)	GPT (iu/l)	LDH (iu/l)
G_1	16.03 ± 4.84	22.61 ± 2.67	24.70 ± 0.06	12.72 ± 1.84	46.72 ± 1.77
G_2	19.27 ± 4.04**	44.13 ± 6.20*	11.70 ± 0.03**	17.67 ± 0.06*	106.70 ± 12.50*

Table 1: *Chemical enzymatic changes. Table (1): Average values (mean ± SD) of serum enzymes of the combined 1% alum and 1% polymer-dosed Newzealand rabbits *denotes P < 0.05 **denotes P < 0.01 ***denotes P < 0.001*

Variable (P < 0.05-0.01) increases in the serum activities of ALP, GOT, GPT and LDH were observed. Significant (P < 0.01-0.001) decreases were observed when testing their serum level of CK compared to the control group. The un-dosed rabbits of group showed the normal activity of the serum enzymes.

Chemical metabolic changes

Group/Dose	Albumin	Urea	Total protein	Cholesterol
G_1	3.85 ± 0.04	30.35 ± 0.69	4.99 ± 0.13	62.37 ± 5.26
G_2	2.70 ± 0.26*	$41.67 \pm 15.88^{N.S}$	$5.33 \pm 0.51^{N.S}$	108.67 ± 12.06*

Table 2: *Chemical metabolic changes. Average values (mean ± SD) of serum metabolites of the combined 1% alum and 1% polymer-dosed Newzealand rabbits.*

Group/Dose	Bilirubin	Glucose	Creatinine
G_1	0.12 ± 0.04	81.13 ± 1.54	1.38 ± 0.01
G_2	0.64 ± 0.29***	$102.00 \pm 16.37^{N.S}$	$1.40 \pm 0.61^{N.S}$

*NS = Not significant *denotes P < 0.05 ***denotes P < 0.001*

The serum of test group revealed decreasing (P < 0.05) albumin and increasing (P < 0.05-0.001) cholesterol and bilirubin concentrations, while serum levels of total protein, glucose and creatinine were not different (P > 0.05) from those of the un-dosed group 1 rabbits. The serum level of urea of group 2 rabbits were not different (P > 0.05) from the normal values obtained from the control un-dosed rabbits. The un-dosed rabbits of group 1 showed the normal serum metabolic values.

Chemical electrolytes changes

Group /Dose	Mg (mg/dl)	Iron (µg/dl)	Na (mg/dl)	K (mg/dl)	Ca (mg/dl)	P (mg/dl)
G_1	1.56 ± 0.17	256.23 ± 39.81	125.41 ± 3.22	5.07 ± 0.12	11.38 ± 0.10	4.34 ± 0.13
G_2	0.39 ± 0.42**	35.93 ± 9.50**	ND	ND	3.17 ± 0.67**	1.52 ± 0.09***

Table 3: *Chemical electrolytes changes. Average values (mean ± SD) of serum electrolytes of the combined 1% alum and 1% polymer-dosed Newzealand rabbits.*
*ND = Not determined ** denotes P < 0.01 *** denotes P < 0.001*

The concentrations of all the electrolytes in the serum of test rabbits showed significant (P < 0.01 - 0.001) decreases, when compared to the control group.

Chemical haematological changes

Group /Dose	Hb (g%)	PCV %	RBCs (x10^6)	WBCs (x10^3)
G_1	10.92 ± 0.83	35.54 ± 4.33	3.79 ± 0.29	3.86 ± 2.90
G_2	11.48 ± 0.74$^{N.S}$	28.40 ± 2.96*	7.18 ± 1.4 9**	9.13 ± 5.03**

Table 4: *Average haematological values (mean ± SD) of the combined 1% alum and 1% polymer-dosed Newzealand rabbits. NS = Not significant *denotes P < 0.05 **denotes P < 0.01*

Haemoglobin concentrations in all test groups were insignificant (P > 0.05) when compared to those of the control group. Values of the PCV of rabbits in groups 2 were decreased (P < 0.01) compared to the control rabbits. The RBCS and WBCs values of rabbits in groups 2s were increased (P < 0.01) in compared to the un-dosed control rabbits. Normal hematological values were recorded in the control group.

Discussion

In this study, the daily oral doses of the blend of 1% polyDADMAC and 1% alum to Newzealand rabbits at the ratio of 1:2 respectively, was a cause of medium mortality. This produced changes in the vital organs and tissues of goats with obvious clinical signs which were mostly pertinent to the stimulation of the central nervous system (CNS) with suggested inhibition of the serum cholinesterase activity which was not-unfortunately measured leading to abnormal posture and gaits and nervous sings due to the action of the organochlorides as a diffuse stimulant of the C.N.S. [3,-5]. Development of diarrhea in the under test group could be due to enteritis and erosion of the intestinal epithelium seen clearly in histopathological sections or due to the increased peristaltic movements probably due to cholinesterase inhibition or to both [6]. Pulmonary disorders manifested by difficulty in respiration, hemorrhages, congestion, oedema and emphysema may be attributed to the direct irritant action of the drug or its metabolites.

The presence of hemorrhages in different organs suggests that the blend is an endotheliotoxic substance, while the wide spread of lymphocyte infiltration in these organs may be reasoned as a reaction to the chemical treatment [7]. The stress caused by the blend dosing caused variable fluctuations in the body weight gains may be due to differences in the food conversion ratios and reduced appetite [8,9].

The significant changes in the activities of ALP, AST, LDH, GPT and CK are suggestive of hepatic excretory dysfunction. This was accompanied by increased concentration of serum bilirubin, centrilobular necrosis and generalized fatty changes indicating hepatic dysfunction and damage [10-16]. Release of some hepatic enzymes into plasma like AST, which is attributed to liver damage [11] or to altered membrane permeability [17], may lead in some instances to muscle shivering and tremors especially if accompanied by the miscellaneous effects of the blend indicated by the decreased levels in all electrolytes .These electrolytes have effects on muscle action, potential and eventual spasms [18,19]. In addition, renal haemorrhages, congestion and necrosis, shrinkage of glomeruli and the arched posture caused by pain are all suggestive of the renal damage caused by the blend or its metabolites [20]. Splenic haemosiderosis may indicate the destruction of erythrocytes judged by the increased values of RBC which might also because of the decrease in the packed cell volume (PCV). Hearts were almost unaffected as well as the Hb concentrations. The white blood corpuscles (WBCs) were increased in animals of group two may be because the blend or its metabolites were pointed out by the animal body as endotoxins [7,21].

Conclusion

Water represents one of the most pollutable natural environmental resources; this entails man-made purification techniques and additives, which is a very rich research-demanding field. Alum and polyDADMAC are approved to be very the conclusion that make their usage for water treatment for drinking purposes vey questionable.

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Volume 1 Issue 3 June 2015