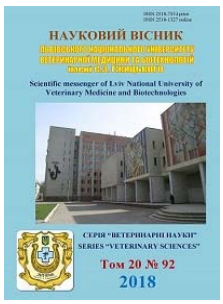

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Comparative Haematological and Biochemical Effects of Cocktail Vaccine (DHLPPi) and *Abrus precatorius* Seed Aqueous Extract on Canine Parvoviral Vaccinated and Unvaccinated Nigerian Local Dogs

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Because of increasing incidence of resistance to infectious microorganisms, immunotherapy has been considered as an alternative/complementary to chemotherapy. More so Abrus precatorius leaf extract has been reported to have immunomodulatory effects in animals. In view of this, comparative haematology and biochemistry of cocktail vaccine (DHLPPi) and Abrus precatorius seed extract (APSE) was studied in rats. Sixteen (16) Nigerian local dogs of both sexes weighing 7.19 ± 0.46 kg, aged 15.44 ± 0.76 weeks old were divided into four groups of 4 each. Group 1, 2, 3 and 4 was administered 1 ml of normal saline (0.9%), APSE (2.72 mg/Kg), APSE (2.72 mg/Kg)+DHLPPi (1 ml) and DHLPPi (1 ml) respectively. Fourteen days after administration of the extract and vaccine, 3 ml of blood sample was collected from each of the dogs for haematology and serum biochemistry. The findings revealed significantly (P < 0.05)decreased packed cell volume, haemoglobin, erythrocytes, neutrophils, leucocytes, platelets and increased monocytes, decreased total protein, albumin, globulin, albumin/globulin ratio, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase in the group administered extract and extract/vaccine. Hence, the extract and extract/DHLPPi vaccine can be used in prevention chronic viral infection of dogs.

Key words: *Abrus precatorius, Vaccine, Immunoinhibition, Canine parvovirus.*

Порівняння гематологічних та біохімічних показників собак при застосуванні вакцини (DHLPPi) та водного екстракту насіння Abrus precatori

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З давніх часів лікарські рослини використовувалися при лікуванні та за для контроля захворювань людини та тварин. Деякі рослини впливають на гематологічні та біохімічні показники сироватки крові. Також існують відомості що деякі сучасні противірусні препарати є неспецифічними для конкретних вірусів. Тому розробка нових економічно ефективних і специфічних противірусних схем є досить актуальним а також першочерговим завданням сучасних досліджень. Через зростання випадків стійкості до інфекційних мікроорганізмів, імунотерапія розглядається як альтернатива хіміотерапії. Більш того, екстракт листя Abrus precatori, як повідомляється, має імуномодулюючу дію у тварин. З огляду на це, нами були проведені дослідження на щурах по визначенню проведенню порівняльної гематологічної та біохімічної оцінки сироватки крові після застосування асоційованої вакцини (DHLPPi) та екстракту з насіння Abrus precatori (APSE). Також для дослідів використовували шістнадцять (16) нігерійських місцевих собак обох статей вагою 7,19 ± 0,46 кг, віком 15,44 ± 0,76 тижнів. Тварин було розділено на чотири групи по 4 у кожній. Групам 1, 2, 3 і 4 вводили відповідно 1мл фізіологічного розчину (0,9%), APSE (2,72 мг/кг), APSE (2,72 мг/кг) + DHLPPi (1 мл) та DHLPPi (1 мл) відповідно. Через чотирнадцять днів після введення екстракту та вакцини від кожної собаки відбирали 3 мл крові для дослідження гематологічних та біохімічних показників сироватки крові. В результаті проведених досліджень вста-

новили що застосування вакцини та екстракту має високу єфективність у профілактиці вірусних захворювань собак. В дослідних группах де проводили комплексне застосування екстракту разом з DHLPPi єфективність профілактики вірусних захворювань набагато вище.

Ключові слова: *Abrus precatorius, вакцина, імуноінгітація, парвовірус собак.*

Introduction

Since ancient times, medicinal plants have been used in the treatment and control of human and livestock diseases and herbal remedies represent the source of one in every four drugs (Garg, 2006). Some plants have effects on hematology and serum biochemistry (Jiang et al., 2015). Viral infections are known as one group of the major causes of death worldwide (Adelowotan et al., 2008). Some antiviral drugs are nonspecific for particular viruses (Bhutia et al., 2009). The development of novel cost-effective and specific antiviral regimens is the prime focus of the current medical research (Adedapo et al., 2007). The plant-related drugs have activities against viral infections (Anam, 2001).

Some medicinal plants used in the treatment of viral diseases in animals include *Acacia nilotica, Gardenia erubescens* (Ganesan and Bhatt, 2008), *Vigna unguiculata* (Guyton and Hall, 2007), *Englerina gabonensis subsp gabonensis and Globimatula globiferus var letuzeyi* used the treatment of FMD cattle. While *Epiphyllum truncata* (Sacks, 2009), *Parkia filicolidea* (Cooper, 2004), *Cannabis indica* (Omage et al., 2015), *Lugenaria vulgeris, Khaya senegalensis,* and *Datura metel* are used in the treatment of Newcastle disease in chicken.

Abrus precatorius Linn is used to cure fever, stomatitis, bronchitis, asthma and diabetes (Newman and Cragg, 2007), chronic nephritis (Saganuwan, 2009), cancer (Saganuwan, 2011), sores, scratches, wounds, leucoderma, tetanus, boils, abscesses, for prevention of rabies (Sacks, 2009), with haematonic and plasma expander effect (Saganuwan, 2010). *A. precatorius* extracts induce apoptosis on various types of cancers (Garg, 2006). The plant has antitumoral (Ramnath et al., 2002), mitogenic (Saganuwan and Gulumbe, 2005b), antifertility (Saganuwan, 2009), immunopotentiating (Ligha et al., 2009), antimicrobial (Newman and Cragg, 2007), immunostimulant (Saganuwan, 2009), antianaphylactic (Sacks, 2009), and anti-inflammatory activities. The present study was undertaken to evaluate the effects of aqueous seed extract of *Abrus precatorius* and DHLPPi vaccine on haematological and biochemical parameters of unvaccinated and vaccinated Nigerian local dogs.

Materials and methods

Collection of Plant material. The plant materials (seeds) used for the study were collected from Kwande Local Government Areas of Benue State, Nigeria between the months of September and November and identified by a botanist in the Department of Biological Science, University of Agriculture, Makurdi where a voucher with voucher number (11) specimen was deposited.

Preparation of Extract. The seeds were thoroughly washed with tap water, dried on filter papers and air dried under open shade for 1 month. The seeds were pulverized with the help of a mortar and pestle to coarse powder and finally ground into fine powder using a grinding machine. Fifty grammes (50 gm) of *Abrus precatorius* seed powder was dissolved in four hundred and fifty milliliter (450 mL) of aqueous extract in a conical flask. The mixture was shaken intermittently throughout a whole day using glass rod stirrer and allowed to stand overnight. The mixture was separately filtered with Whatman filter paper No. 1 into measuring cylinder and concentrated at 60ºC in an incubator and stored in a refrigerator at 4 ºC until required for use (Sacks, 2009).

Administration of Abrus Precatorius seed extract and vaccine. The method of Yamba was used for selection of extract doses used for haematology and serum biochemistry. Median lethal dose (LD_{50}) of 187.5 mg/Kg body weight of the extract was extrapolated to dogs (2.72 mg/Kg) using Human Equivalent Dose (HED) formula. The extract was prepared as 0.5% solution and administered orally to all the dogs before feeding, for a period of fourteen (14) days. The dogs were divided into four groups of 4 each. Group 1 served as the control and received normal saline, group 2 received the 2.72 mg/Kg aqueous extract alone and group 3 dogs were vaccinated against canine parvovirus infection using DHLPPi in addition to 2.72 mg/Kg of the extract whereas group 4 dogs were vaccinated with DHLPP vaccine only. Blood sample (3 ml) was obtained before and after the treatment from the cephalic vein with the help of 21 gauge needle and 10mL syringe for the determination of hematological and biochemical parameters.

Haematological Parameters. Haematological parameters were determined according to the method of Cheesbrough. The parameters were red blood cells (RBCs) count, packed cell volume (PCV), haemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBCs) count and differential white blood cells count (DWBC).

Serum Biochemical Parameters. Serum biochemical parameters were determined. The parameters were total protein, albumin, globulin, albumin/globulin ratio, alkaline phosphatase (ALP) and alanine aminotransferases (ALT), aspartate aminotransferases (AST), potassium, sodium and chloride.

Statistical Analysis. The data on haematological and serum biochemical parameters were expressed as mean ± standard error of mean (SEM). One way analysis of variance (ANOVA) was used to analyze the data on haematological and serum biochemical parameters at 5% level of significance (Saganuwan and Onyeyili, 2010).

Results

The haematological changes observed in dogs administered aqueous seed extract of A. precatorius,

extract and DHLPPi vaccine and DHLPPi vaccine only are presented in Table 1. Dogs administered 2.72 mg/Kg of the extract, 2.72 mg/Kg of the extract + 1 dose of DHLPPi vaccine and 1 dose of DHLPPi vaccine showed significant decrease ($P < 0.05$) in PCV and HB relative to the control (normal saline 0.9%) and the pretreatment group.

The leucogram showed a statistically significant decrease ($P < 0.05$) in WBC count and NEU in dogs given 2.72 mg/Kg of extract relative to the control and pretreatment group. While a significant increase ($P < 0.05$) was observed for lymphocyte counts in dogs given 2.72 mg/Kg extract, 2.72 mg/Kg extract + DHLPPi and DHLPPi respectively.

A significant decrease ($P < 0.05$) was seen in MON in dogs given 2.72 mg/Kg extract and 2.72 mg/Kg extract + DHLPPi and EOS in dogs given 2.72 mg/Kg extract + DHLPPi group, while a significant decrease ($P < 0.05$) was observed in dogs given DHLPPi vaccine group respectively.

Table 1
The effects of aqueous seed extracts of *A. precatorius* extract and DHLPPi vaccine on haematological parameters of dogs

Parameters	Group	Normal Saline (0.9%)	Extract (2.72 mg/kg)	Extract (2.72 mg/kg) + DHLPPi vaccine	DHLPPi vaccine
PCV (%)	Pretreatment	36.75 ± 1.49	37.00 ± 2.12	38.50 ± 3.66	37.25 ± 2.78
	Treatment	38.00 ± 1.22	32.00 ± 1.41[b]	32.10 ± 0.58[b]	31.00 ± 1.73[b]
HB (g/L)	Pretreatment	12.25 ± 0.49	12.30 ± 0.71	12.80 ± 1.22	12.43 ± 0.92
	Treatment	12.65 ± 0.39	10.68 ± 0.47[b]	10.67 ± 0.20[b]	10.30 ± 0.58[b]
RBC ($X10^9$/ L)	Pretreatment	6.12 ± 0.25	6.16 ± 0.35	6.42 ± 0.61	6.21 ± 0.46
	Treatment	6.33 ± 0.20	5.33 ± 0.24[b]	5.33 ± 0.10[b]	5.11 ± 0.24[b]
MCV (fL)	Pretreatment	60.00 ± 0.00	59.00 ± 0.00	59.50 ± 0.29	60.00 ± 0.00
	Treatment	60.00 ± 0.00	60.00 ± 0.00	60.00 ± 0.00	60.33 ± 0.33
MCH (pg)	Pretreatment	19.25 ± 0.48	19.25 ± 0.25	19.25 ± 0.25	19.00 ± 0.41
	Treatment	19.22 ± 0.25	19.5 ± 0.50	19.00 ± 0.58	19.33 ± 0.33
MCHC (g/dL)	Pretreatment	32.25 ± 0.48	32.5 ± 0.29	32.75 ± 0.85	32.00 ± 0.58
	Treatment	31.75 ± 0.25	32.5 ± 0.50	32.00 ± 0.58	32.00 ± 0.00
WBC ($X10^9$/ L)	Pretreatment	10.88 ± 0.56	12.05 ± 0.75	9.55 ± 0.85	9.65 ± 1.08
	Treatment	9.80 ± 1.00	8.85 ± 0.67[b]	9.41 ± 1.14[b]	9.67 ± 0.75
NEUT ($X10^9$/ L)	Pretreatment	70.00 ± 3.42	65.25 ± 2.43	68.75 ± 2.57	67.00 ± 3.94
	Treatment	70.25 ± 3.66	64.00 ± 4.12[b]	65.67 ± 2.03[b]	67.33 ± 3.71
LYM ($X10^9$/ L)	Pretreatment	21.25 ± 3.07	28.75 ± 1.11	27.50 ± 2.26	29.75 ± 1.18
	Treatment	22.5 ± 2.99	29.50 ± 1.85[a]	29.33 ± 1.20[a]	30.33 ± 2.85[a]
MON ($X10^9$/ L)	Pretreatment	8.00 ± 2.71	5.25 ± 1.11	3.50 ± 0.87	7.25 ± 2.18
	Treatment	6.75 ± 2.99	6.00 ± 2.48[a]	4.67 ± 2.40[a]	2.00 ± 1.16[b]
EOS ($X10^9$/ L)	Pretreatment	0.75 ± 0.75	0.75 ± 0.48	0.55 ± 0.25	0.50 ± 0.29
	Treatment	0.50 ± 0.50	0.50 ± 0.50	0.50 ± 0.00	0.53 ± 0.33
BAS ($X10^9$/ L)	Pretreatment	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Treatment	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
PLT ($X10^9$/ L)	Pretreatment	176.8 ± 4.03	174.3 ± 2.18	181.50 ± 4.65	192.8 ± 11.69
	Treatment	175.3 ± 3.68	172.8 ± 2.50[b]	176.3 ± 6.89[b]	179.3 ± 10.35[b]

Key: a = significantly higher ($P < 0.05$) in comparison with the control; b = significantly lower ($P < 0.05$) in comparison with the control; RBC = Red Blood Cells, HB = Hemoglobin, PCV = Packed Cell Volume, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, WBC = White Blood Cells, LYM = Lymphocytes, MON = Monocytes, NEU = Neutrophils, EOSIN = Eosinophils, PLT = Platelets, DHLPPi = Distemper, Hepatitis, Leptospirosis, Parvovirus and Para-influenza vaccine.

Dogs given 2.72 mg/Kg extract + DHLPPi and DHLLPi showed a significant decrease ($P < 0.05$) in PLT respectively.

The serum biochemical changes observed in dogs administered aqueous seed extract of A. precatorius extract and DHLPPi vaccine and DHLPPi vaccine only are presented in Table 2.

Dogs given 2.72 mg/Kg of the extract, 2.72 mg/Kg of the extract + 1 dose of DHLPPi vaccine and 1 dose of DHLPPi vaccine showed significant decrease ($P < 0.05$) in total protein respectively. A significant decrease ($P < 0.05$) in the albumin, the group treated with 2.72 mg/Kg extract + DHLPPi, DHLPPi and extract (2.72 mg/Kg) respectively. Enzyme assay showed a significant decrease ($P < 0.05$) in ALP of dogs administered 2.72 mg/Kg extract, 2.72 mg/Kg of the extract + DHLPPi and DHLPPi. There was a significant AST decrease ($P < 0.05$) in 2.72 mg/Kg extract and 2.72 mg/Kg extract + DHLPPi. A significant decrease ($P < 0.05$) in ALT was observed in dogs that received extract (2.72 mg/Kg), 2.72 mg/Kg extract + DHLPPi and DHLPPi.

Electrolyte assay showed a significant increase ($P < 0.05$) in potassium of dogs that received 2.72 mg/Kg extract and 2.72 mg/Kg extract + DHLPPi group, while sodium was significantly decreased ($P < 0.05$) in DHLPPi group.

Dogs given 2.72 mg/Kg of the extract, 2.72 mg/Kg of the extract + 1 dose of DHLPPi vaccine and 1 dose of DHLPPi vaccine showed significant decrease ($P < 0.05$) in total protein respectively.

A significant decrease ($P < 0.05$) in the albumin, the group treated with 2.72 mg/Kg extract + DHLPPi, DHLPPi and extract (2.72 mg/Kg) respectively.

Enzyme assay showed a significant decrease ($P < 0.05$) in ALP of dogs administered 2.72 mg/Kg extract, 2.72 mg/Kg of the extract + DHLPPi and DHLPPi. There was a significant AST decrease ($P < 0.05$) in 2.72 mg/Kg extract and 2.72 mg/Kg extract + DHLPPi. A significant decrease ($P < 0.05$) in ALT was observed in dogs that received extract (2.72 mg/Kg), 2.72 mg/Kg extract + DHLPPi and DHLPPi. Electrolyte assay showed a significant increase ($P < 0.05$) in potassium of dogs that received 2.72 mg/Kg extract and 2.72 mg/Kg extract + DHLPPi group, while sodium was significantly decreased ($P < 0.05$) in DHLPPi group.

Table 2.
The effects of aqueous seed extracts of *A. precatorius* extract and DHLPPi vaccine on serum biochemical parameters of dogs

Parameters	Group	Normal Saline (0.9%)	Extract (2.72 mg/kg)	Extract (2.72 mg/kg) + DHLPPi vaccine	DHLPPi vaccine
Total protein (mg/dL)	Pretreatment	6.05 ± 0.36	5.58 ± 0.33	5.38 ± 0.28	5.40 ± 0.27
	Treatment	6.25 ± 0.19	4.50 ± 0.60[b]	4.63 ± 0.68[b]	4.90 ± 0.66[b]
Albumin (mg/dL)	Pretreatment	3.63 ± 0.67	2.90 ± 0.09	2.9 ± 0.22	2.85 ± 0.30
	Treatment	3.60 ± 0.34	2.20 ± 0.38[b]	2.43 ± 0.38[b]	2.40 ± 0.72[b]
Globulin (mg/dL)	Pretreatment	2.42 ± 0.31	2.68 ± 0.24	2.48 ± 0.06	2.55 ± 0.03
	Treatment	2.45 ± 0.15	2.30 ± 0.22[b]	2.20 ± 0.30[b]	2.50 ± 0.06
Albumin/Globulin Ratio	Pretreatment	1.50 ± 0.00	1.08 ± 0.00	1.17 ± 0.00	0.94 ± 0.00
	Treatment	1.47 ± 0.00	0.56 ± 0.00 [b]	0.61 ± 0.00[b]	0.96 ± 0.00
ALP (u/L)	Pretreatment	61.5 ± 3.86	15.0 ± 2.65	17.25 ± 1.38	40.0 ± 13.19
	Treatment	61.75 ± 3.28	14.5 ± 2.22[b]	14.0 ± 5.51[b]	30.67 ± 13.35[b]
AST (u/L)	Pretreatment	12.75 ± 5.23	7.00 ± 1.23	7.50 ± 0.87	12.95 ± 3.57
	Treatment	12.85 ± 5.20	7.75 ± 1.55[a]	11.67 ± 3.18[a]	13.03 ± 4.98
ALT (u/L)	Pretreatment	11.75 ± 2.29	7.25 ± 1.60	6.25 ± 0.95	6.95 ± 1.44
	Treatment	11.80 ± 2.52	9.0 ± 1.29[a]	13.67 ± 3.18[a]	7.00 ± 1.53
Potassium (mmol/L)	Pretreatment	3.78 ± 0.22	3.05 ± 0.49	2.83 ± 0.45	3.60 ± 0.23
	Treatment	3.55 ± 0.22	4.98 ± 1.08[a]	4.43 ± 1.27[a]	3.58 ± 1.13
Sodium (mmol/L)	Pretreatment	139.3 ± 13.30	167.3 ± 16.9	149.3 ± 5.02	130.8 ± 9.01
	Treatment	134.8 ± 12.50[b]	107.0 ± 4.66[b]	109.3 ± 28.47[b]	116.3 ± 16.84[b]
Chloride (mmol/L)	Pretreatment	106.5 ± 3.66	89.5 ± 5.39	117.5 ± 4.03	106.0 ± 2.80
	Treatment	103.3 ± 2.72[b]	85.75 ± 1.65[b]	109.0 ± 13.32[b]	89.0 ± 5.57[b]

Key: a = significantly higher ($P < 0.05$) in comparison with the control; b = significantly lower ($P < 0.05$) in comparison with the control; ALP = Alkaline Phosphatase, ALT = Alanine Aminotransferase, AST = Aspartate Aminotransferase DHLPPi = Distemper, Hepatitis, Leptospirosis, Parvovirus and Para-influenza vaccine

Discussion

The significant decrease in PCV, HB and RBC in dogs administered 2.72 mg/Kg extract, 2.72 mg/Kg extract + DHLPPi and DHLPPi show that both the extract and the vaccine could cause hemolysis in Nigerian local dogs. This disagrees with the finding that the extract causes increase in PCV, HB and RBC (Saganuwan and Gulumbe, 2005b). Hence the prolong administration of the seeds to animals may cause anemia. Saganuwan and Onyeyili (2016) and Adedapo et al. (2007) had earlier reported that Abrus precatorius extract could cause haemolysis. The plant's active principle is abrin, a toxalbumin (phytoprotein), which is antigenic and may be responsible for the observed toxic effects (Saganuwan and Ogalue, 2008). Abrin consists of abrus agglutinin (a haemaglutinin), and toxic lectins abrins a–d, the toxic glycoproteins present in the seeds (Sacks, 2009). The leucopenia and neutropenia observed in the present study show that both the plant and the extract have immunoinhibitory effect and as such can be used in the treatment of hyperimmuno stimulatory diseases such as systemic lupus erythematosus, psoriasis and inflammatory bowel disease by causing decreasing immune response (Saganuwan and Gulumbe, 2005a). Lymphocytosis observed in all the treatment groups showed that the extract and vaccine have immunomodulatory effects. This could be due to stimulation of humoral system (Ligha et al., 2009). Although, vaccine induced immunosuppression had been reported in humans (Omage et al., 2015), polyvalent canine vaccines cause significant lymphopenia to mitogen about 3–11 days after vaccination. By day 14 post vaccination, the lymphocytopenia changes lymphocytosis (Saganuwan and Onyeyili, 2016) which is beneficial during immune response.

The observed lymphocytosis could be effect of the active components of the extract and vaccine on the body defense mechanism (Saganuwan and Gulumbe, 2005b). Selective enhancement of the immune response is a primary goal in prevention of diseases. Drugs and chemicals have been known to induce lymphocytosis in man and animals (Sacks, 2009).

The monocytosis observed in the present study disagrees with the report of Saganuwan et al. (2014) indicating that Abrus precatorius extract caused monocytopenia. The cause of immune-stimulatory/inhibitory potential of the A. precatorius seed extract may be unknown. Hypoproteinaemia, hypoalbuminaemia observed in all the groups and low albumin-globulin ratio seen in extract and extract +

DHLPPi suggest the immunomodulatory effects (Saganuwan and Ogalue, 2008). The significant decrease in serum ALP observed might be due to kidney damage because decreased level of ALP is associated with severe chronic nephritis. The increase in serum ALT and AST observed in the extract and extract + vaccine (DHLPPi) groups respectively is suggestive of liver involvement. ALT is mainly found in hepatic tissue whereas AST occurs in large concentration in cardiac as well as hepatic tissues with small amount present in other tissues (Saganuwan and Onyeyili, 2010). The finding agrees with the report of Willard et al. (1999) and Saganuwan and Ogalue (2008) indicating that ALT and AST are liver enzyme markers that could be used to indicate hepatic damage. Hyperkalemia was observed in the extract and the extract + vaccine (DHLPPi) disagrees with the findings of Saganuwan and Onyeyili indicating that Abrus precatorius extract could cause hyperkalemia. Hyponatremia and hypochloremia observed in the present study could be due to the renal damage as primary renal diseases allow the escape of the element through the urine (Saganuwan and Onyeyili, 2010). Decreased level of sodium could also be as a result of increased water gain or loss of sodium. Serum chloride regulates blood pressure, fluid and electrolyte balance, gastric fluid, and chloride shift in HCO_3^- transport in erythrocytes as such severe depletion of serum chloride levels (hypochloremia) can cause metabolic alkalosis characterized by mental confusion, apnea, paralysis, muscle spasm. Care may however be taken as prolong use of the plant may result to these effects (Saganuwan et al., 2014).

Conclusion

Aqueous extract of *Abrus precatorius* seed has immunomodulatory potentials and may be used as blood tonic and as an immune-stimulant in anemic and immune-compromised diseased conditions like chronic viral infections.

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