Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2017.384.391

Research Article

Effects of Alkaloid Rich-Fraction (ARF) of Methanol Extract of Ricinus Communis (RC) Seeds on Immune Responses, Inflammatory Reactions and Liver Functions

[1]Njoku Ugochi Olivia, [1]Nwodo Okwesili Fred Chiletugo and [2]Uroko Robert Ikechukwu

[1]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria
[2]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Abstract

Background and Objective: *Ricinus communis* seeds are widely consumed for nutritional and medicinal benefits. This study investigated the effects of Alkaloid-Rich Fraction (ARF) of methanol extract of *Ricinus communis* seed on inflammatory reactions, immune response and its hepatoprotective potentials on male wistar albino rats challenged with CCl_4 in order to maximize its health benefits. **Materials and Methods:** *In vitro* study was employed in determining the effect of ARF on the hemolytic activity of complement proteins and immunostimulatory activity. The effects of ARF on immune response and hepatoprotective potentials were carried out using male Wistar albino mice and rats challenged with CCl_4 in corn respectively. **Results:** The ARF of *Ricinus communis* seed caused significant ($p<0.05$) increase in the phagocytic index and stimulated phagocytosis in a concentration dependent manner when compared to the control. Complement protein activity significantly ($p<0.05$) increased hemolysis of the sensitized sheep red blood cells in a concentration-dependent manner when incubated in the ARF indicating that the ARF could be used in prevention of infections associated with complement protein deficiency. A dose dependent significant decrease ($p<0.05$) in the delayed type hypersensitive response was observed while significant increase ($p<0.05$) in the primary and secondary antibody titers of the test groups relative to normal control were also observed. However, there was significant decrease ($p<0.05$) in both primary and secondary titer values when compared to the standard control that received 2.5 mg kg^{-1} of levamisole. At higher dose of the ARF, aspartate aminotransferase activity significantly ($p<0.05$) increased when compared with the control. In addition, alanine aminotransferase and alkaline phosphatase activities of the treated groups significantly decreased ($p<0.05$) when compared to positive control indicating that the ARF could maintain liver integrity and functions. **Conclusion:** The findings of this study suggest that the ARF has hepatoprotective potentials and positive effects on immune system coupled with potentials anti-inflammatory activity that could help the body defense system, fight diseases and pathogenic organisms.

Key words: *Ricinus communis* seeds, hepatoprotection, alkaloid-rich fraction, inflammatory reactions, liver marker enzymes, immune system

Received: March 16, 2017 Accepted: June 30, 2017 Published: July 15, 2017

Citation: Njoku Ugochi Olivia, Nwodo Okwesili Fred Chiletugo and Uroko Robert Ikechukwu, 2017. Effects of Alkaloid Rich-Fraction (ARF) of methanol extract of Ricinus Communis (RC) seeds on immune responses, inflammatory reactions and liver functions. J. Applied Sci., 17: 384-391.

Corresponding Author: Uroko Robert Ikechukwu, Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria Tel: +2348065914471

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ricinus communis is a perennial shrub of the *Euphorbiaceae* family[1]. It is a tropical plant, commonly known as castor bean, the palm of Christ and it is distributed widely across the globe[1]. The plant is native to India and is found in the South-Eastern part of Nigeria where it is cultivated for use as a food seasoning. Castor seed is the source of castor oil, which has a wide variety of uses. *Ricinus communis* plant has been used for the treatment of inflammation, liver disorders and also possesses free radical scavenging activity[2-3]. Stems of *Ricinus communis* have been reported to possess anticancer, antidiabetic, diuretic and antiprotozoal activity[4]. It has also been shown that *Ricinus communis* Linn has anti-tubercular activity and antiasthmatic activity[5]. The leaf, root and seed oil of this plant possess hypoglycemic activity Kensa and Syhed, antifertility properties Manpreet, laxative and immunomodulatory activities[6-8].It has also been reported that methanolic extract of *Ricinus communis* seeds possesses antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* [9]. The study carried out by Nwodo *et al* demonstrated that methanol extract fraction of *R. communis* seeds possesses high anti-hyperglycemic effect close to that of metformin and potentials for effective maintenance of lipid profile[10]. It has also been reported by Joshua *et al* that methanol extract of *R. communis* possesses high antioxidant activity which could prevent oxidative stress[11].

Many medicinal plants have been used in the treatment of various diseases due to their immunomodulatory properties and rich sources of bioactive components with curative properties needed in the production of some chemotherapeutic agents[12]. Immune system or function could be enhanced or suppressed by bioactive components in medicinal plants and their products[12]. Medicinal plants can provide an alternative to conventional chemotherapy for a variety of disease especially when host defense mechanism has to be altered. It is now known that various extracts of *Ricinus communis* seeds could be used for the treatment of various diseases and medical conditions. However, the effects of alkaloids (isolated from methanol extract of *Ricinus communis* seeds) or the alkaloid rich fraction on the immune system, inflammatory reactions and liver functions have not been fully investigated. In the current study, the effects of alkaloid rich fraction of methanol extract of *Ricinus communis* seed on immune responses, inflammatory reactions and liver marker enzymes were investigated.

MATERIALS AND METHODS

Chemicals and reagents: All the chemicals and reagents used in this study were of analytical grade and sourced from chemical stores at Nsukka, Enugu State Nigeria and Department of Biochemistry, Faulty of Biological Sciences, University of Nigeria Nsukka, Nigeria.

Plant material: *Ricinus communis* seeds were collected from local areas of Ibagwa, Nsukka and authenticated by Mr. A. Ozioko of the International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State, Nigeria.

Animals: Sixteen male Wistar albino mice and 20 Wistar male albino rats used in this study were obtained from the Animal House of the Department of Zoology, Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatized to laboratory conditions for 1 week under standard conditions of 12 h light and dark cycles. The animals were fed with standard Grower's Mash rats' pellets and water.

Microorganisms and antigen: Pure isolates of *Candida albicans* were obtained from the Mycology Unit of the Department of Microbiology, University of Nigeria, Nsukka and maintained in pure culture prior to use *in vitro* immunostimulant activity. While Sheep Red Blood Cells (SRBC) obtained from the Faculty of Veterinary Medicine of the same university were used to induce delayed type hypersensitivity response in rats.

Methods: The study was carried out between April 17, 2015 and February 8, 2016 at the Department of Biochemistry, Faculty of Biological Sciences; University of Nigeria Nsukka, Nigeria.

Processing of plant material: The *Ricinus communis* seeds were crushed with a Creston high-speed grinder into coarse size. Whole seeds were packed in the grinding chamber, ground, re-weighed and stored in plastic containers.

Fractionation: A known quantity of the pulverized seeds of RC (2 kg), placed in a glass stoppered round bottom flask were soaked in 5 volume chloroform-methanol (2:1), shaken vigorously and allowed to stand for 18 h at room temperature. The filtrate through Whatman No. 4 filter paper was washed with 20% volume of distilled water in a separating funnel resulting in 2 distinct layers. The upper phase (aqueous

methanol layer) was drawn out, evaporated to dryness and weighed. The remaining fraction was fractionated using Sephadex G-15 and various aliquots from the fractions were spotted on (20×20 cm) activated chromatoplates pre-coated with silica gel $F_{254/366.}$ The plates were developed in chromatographic tanks pre-equilibrated with butanol-acetic acid-water (65:13:22) as the solvent system, for 1 h and air dried. Fractions whose samples yielded an orange on violet color (positive for alkaloids) were pooled as the alkaloid rich fraction and screened for immunomodulatory properties.

Determination of *in vitro* immunostimulant activity: In order to study the effect of alkaloid-rich fraction of *Ricinus communis* seed on phagocytic function of Polymorphonuclear Neutrophils (PMN), *in vitro* slide culture technique was adopted[13-14]. Each slide was observed under 100× oil immersion objective and the extent of phagocytosis evaluated by the method described by Ganachari *et al* using *Candida albicans*[14].

Determination of effect of the alkaloid rich fraction on hemolytic activity of complement protein: The effect of the alkaloid- rich fraction on the hemolytic activity of complement system was investigated *in vitro* by a modified version of a microtiter method Sharma *et al*.[15] and Garbacki *et al*.[16].

***In vivo* study (Phase I):** The *in vivo* study of the effect of the extract on delayed type hypersensitivity response and Humoral Antibody (HA) response were carried out using 20 male albino rats divided into 5 groups of 4 rats each was employed in this study. The control (Group 1) received 0.2 mL of normal saline, Groups 2, 3 and 4 were each administered (p. o) 50, 100 and 200 mg kg^{-1} b. w of the alkaloid rich fraction respectively while Group 5 was treated with 2.5 mg kg^{-1} of levamisole (standard drug). The administration was done once daily for 72 h prior to sensitization and continued daily till the challenge.

Delayed type hypersensitivity response: The method described by Sharma *et al* was adopted to assess Sheep Red Blood Cell (SRBC) induced Delayed Type Hypersensitivity (DTH) response in mice[15].

Humoral Antibody (HA) response: Primary antibody titer was estimated on day 7 (prior to challenge) and secondary titer on day 14 using the standard haemagglutination test[15,17].

***In vivo* study (Phase II):** Effect of the alkaloid rich fraction on liver marker enzymes was investigated using 16 Wistar albino mice which were divided into 4 groups of 4 mice each. After acclimatization, the liver cells were challenged with carbon tetrachloride (CCl_4) in corn oil (3:1 v/v) as a vehicle administered intraperitoneally to the rats. However, normal saline and the alkaloid-rich fraction of *RC* seeds methanol extract were administered orally. The negative control (Group 1), received normal saline for 10 days, on the 8th day, the animals were administered corn oil intraperitoneally. The positive control (Group 2), received normal saline for 8 days and was challenged with 2 mL kg^{-1} b.wt. of CCl_4 in corn oil (3:1), then normal saline was administered for 2 more days. Group 3 received 100 mg kg^{-1} of the alkaloid-rich fraction of RC seeds methanol extract for 8 days, on the 8th day, 2 mL kg^{-1} b.wt. of CCl_4 in corn oil was administered, then the extracts were administered for 2 more days. Group 4 received 200 mg kg^{-1} b.wt. of an alkaloid-rich fraction of RC seeds methanol extract, on the 8th day CCl_4 in corn oil was administered, then extract was administered for 2 more days. Corn oil was used as a standard dosing vehicle to delay the absorption of CCl_4. After the 10th day, the animals were sacrificed after 18 h fasting and the blood collected for assay.

Assay of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities: Randox commercial enzyme kit according to the methods described by Reitman and Frankel[18] were used to assay for AST, ALT and ALP activities.

Statistical analysis: Results obtained were expressed as a Mean±Standard Deviation and analyzed using one-way analysis of variance (ANOVA) and Fisher LSD post hoc test. Differences between means of treated and control groups were considered significant at $p<0.05$. The Statistical Products and Service Solutions (IBM SPSS Statistics 20) were used[19].

RESULTS

Percentage yield: The extraction of 2 kg pulverized seeds of RC and subsequent fractionation gave a percentage yield of 42 g (2.1%) of alkaloid rich fraction that was used for the study. In the evaluation of the mean number of *Candida albicans* cells phagocytosed by Polymorphonuclear Neutrophils (PMNs) on the slide, the stimulation of phagocytosis of *Candida albicans* by the control treatment was comparatively lower, with a phagocytic index of 20.00±0.50. Relative to the control, ARF produced a remarkable effect on the phagocytic activity of Polymorphonuclear Neutrophils (PMNs) (Table 1). Alkaloid rich fraction of *Ricinus communis* seeds

significantly ($p<0.05$) increased the phagocytic index; stimulating phagocytosis. However, at low concentrations the phagocytic activity by PMNs was significantly inhibited. The alkaloid rich fraction exhibited PMN-induced phagocytic activity being inhibitory at low doses but stimulatory at higher doses.

The control sample showed a relatively low optical density when the hemolytic activity of complement proteins was measured. ARF significantly ($p<0.05$) decreased the optical density of the medium as the concentrations increased relative to the control and hence, inhibiting greatly the hemolytic activity of the complement proteins on the sensitized SRBC as shown in Fig. 1.

Table 1: Effect of alkaloid-rich fraction of RC seeds methanol extract on percentage phagocytosis stimulation of polymorphonuclear neutrophils

Treatment	Concentration μg mL^{-1}	Phagocytic index	Phagocytosis stimulation (%)
ARF	100	30.00	50.50*
	50	27.00	35.50*
	25	24.00	20.00*
	12.5	17.47	-12.65*
	6.25	18.30	-8.50*
Control		20.00	0.00

Negative sign (-) indicates inhibition. * shows $p<0.05$ and implies significance

Among the control animals treated with SRB cells prepared in normal saline, the mean Delayed Type Hypersensitivity (DTH) response measured by the increase in the rat paw volume (oedema) was relatively high. Treatment with ARF produced a significant ($p<0.05$) dose dependent inhibition of the DTH response when compared to the control in an inverse manner (Fig. 2). The inhibitory effect of ARF at 50 mg kg^{-1} body weight was comparable to that of the standard drug, Levamisole.

The control group that received normal saline showed a comparatively low mean primary and secondary Antibody (AB) titer values. The group treated with the standard drug levamisole showed significantly ($p<0.05$) increased antibodies in both the primary and secondary antibody response when compared to the control as shown in Fig. 3. Relative to the control, the test groups showed significant ($p<0.05$) increase in both the primary and secondary humoral antibody titer. At the lowest tested dose of 50 mg kg^{-1} body weight, both the primary and secondary antibody titer values were observed to be maximum and also found to be significant ($p<0.05$) when compared to the group treated with the standard immunostimulatory drug (levamisole).

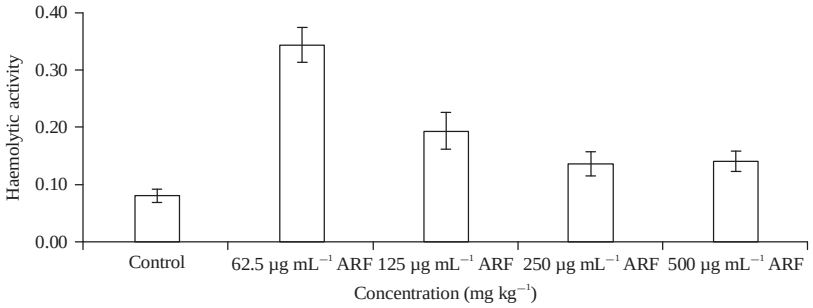

Fig. 1: Inhibitory effects of Alkaloid Rich-Fraction (ARF) of methanol extract of *Ricinus communis* (RC) on hemolytic activity of complement proteins
Each bar represent Mean±Standard Deviation of haemolytic activity

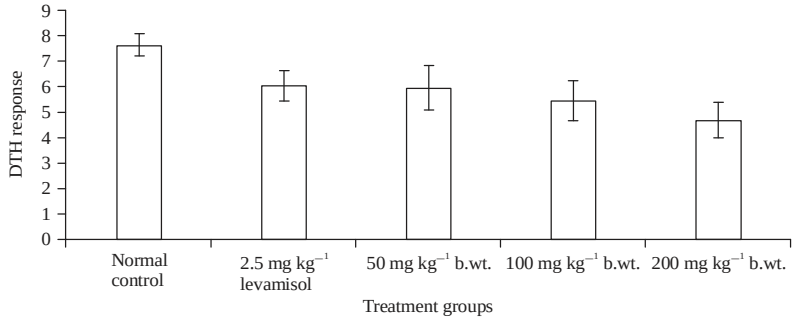

Fig. 2: Inhibitory effect of the Alkaloid Rich-Fraction (ARF) of methanol extract of *Ricinus communis* (RC) on DTH response
Each bar represent Mean±Standard Deviation of DTH response

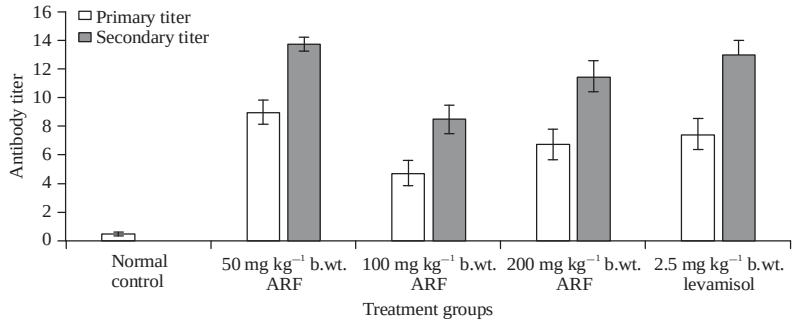

Fig. 3: Stimulation of antibody titer caused by the Alkaloid Rich-Fraction (ARF) of methanol extract of *Ricinus communis* (RC)
Each bar represent Mean±Standard Deviation of antibody titer

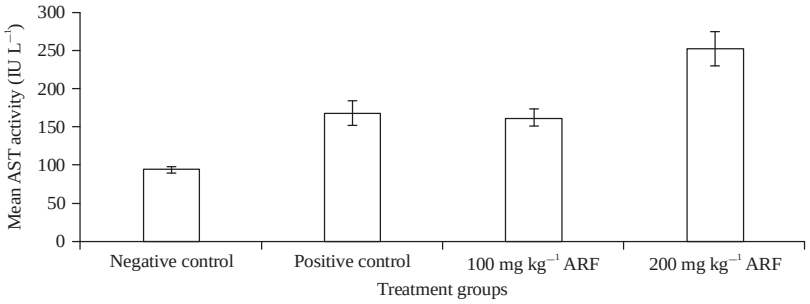

Fig. 4: Effect of the Alkaloid Rich-Fraction (ARF) of methanol extract of *Ricinus communis* (RC) on AST activity of male Wistar albino rats
Each bar represent Mean±Standard Deviation of AST activity

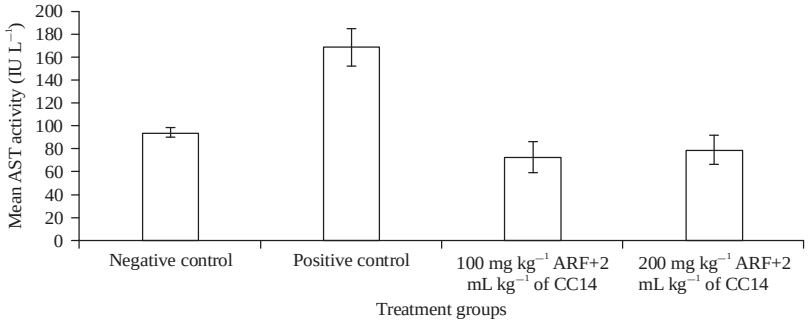

Fig. 5: Effect of the Alkaloid Rich-Fraction (ARF) of methanol extract of *Ricinus communis* (RC) on ALT activity of male Wistar albino rats
Each bar represent Mean±Standard Deviation of ALT activity

The positive control group challenged with 2 mL kg^{-1} body weight of CCl_4 but untreated, showed a significant ($p<0.05$) increase in aspartate aminotransferase (AST) activity relative to the control group that received only the vehicle. Amongst the tested groups challenged with 2 mL kg^{-1} b.wt. of CCl_4, the ARF at 100 mg mL^{-1} produced a non-significant ($p>0.05$) decrease in AST activity when compared to the positive group while at a dose of 200 mg kg^{-1} b.wt., the activity of the AST increased significantly ($p<0.05$) when compared to that of the control and the group administered 100 mg kg^{-1} body weight by ARF as shown in Fig. 4.

Considering the groups induced liver damage with CCl_4, the positive control (group induced but not treated) the ALT activity was observed to be higher when compared to the tested groups treated with the ARF Sample. The activity of ALT at 100 mg kg^{-1} body weight was observed to be lower when compared to the effect of ARF at the dose of 200 mg kg^{-1} body weight. Hence, indicating that the effective dose of ARF may be at 100 mg kg^{-1} b.wt. or lower as shown in Fig. 5.

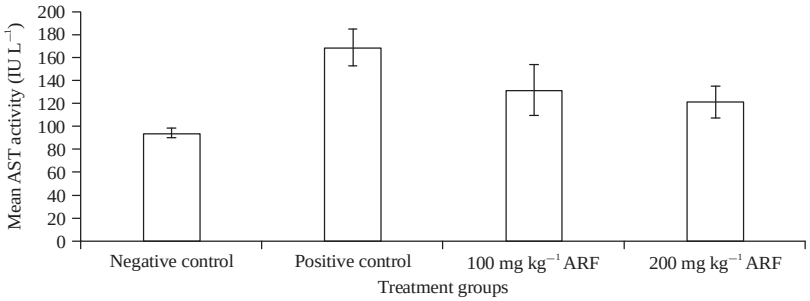

Fig. 6: Inhibitory effect of alkaloid rich fraction of methanol extract of *R. communis* seeds on alkaline phosphatase
Each bar represent Mean±Standard Deviation of ALP activity

There was a significant increase ($p<0.05$) in the alkaline phosphatase activity of positive control when compared to negative control while the induced tested groups treated with ARF doses. The effects of ARF at the tested doses were shown to be reduced when compared to that of the positive control as shown in Fig. 6.

DISCUSSION

The study investigated the effects of Alkaloid-Rich Fraction (ARF) of *Ricinus communis* seed methanol extract on inflammatory reactions, immune response and hepatoprotective potential of liver cells of male Wistar albino rats challenged with CCl_4 using liver marker enzymes as indicators.

The observed significant ($p<0.05$) phagocytic stimulation by 50.50% at the highest tested concentration, 100 μg mL^{-1} revealed the immunostimulatory property of the alkaloid rich fraction. However, the low phagocytic index, observed at concentrations of 6.25 and 12.5 μg mL^{-1}, when compared to basal control revealed immunosuppression. The immunostimulatory effect of the ARF on phagocytic activity of PMN neutrophils may be due to the activation of sarcoma kinases, which catalyze the phosphorylation of the tyrosine residue of the immunoreceptor tyrosine-based activating motif, hence, generating a cascade of signals within the cells that commence phagocytosis. This is in consonance with the earlier finding by Joshi *et al* that the activation of Src kinase is critical for FcγR-mediated phagocytosis[20]. As recorded, high concentrations of the ARF significantly ($p<0.05$) stimulated phagocytosis while low concentrations inhibited phagocytosis. It is possible that the ARF may contain more than one bioactive agent that affect the phagocytic process as suggested by Njoku and Nwodo in their report on the immunomodulatory potential of ARF of *Abrus precatorius* seeds methanol extract[21]. This also agrees with findings of Kumar *et al* who studied the immunomodulatory activity of *Ricinus communis* and reported that the presence of tannins improved phagocytosis of microorganisms[22]. It improved the immune responsiveness against pathogens by activating the non-specific immune system.

The significant ($p<0.05$) increase observed in the degree of hemolysis of the sensitized sheep red blood cells in a concentration-dependent was an indication of immune-stimulatory effect of the alkaloid rich fraction. This may be attributed to a stimulatory effect of the ARF on C4, a central component of the classical pathway of the complement activation leading to the generation of more activated complement proteins. These proteins opsonise pathogens for engulfment by phagocytes while acting as chemo-attractants to recruit more phagocytes on the sites of complement activation and so this alkaloid rich fraction *Ricinus communis* seeds may reduce susceptibility to infections associated with deficiency of complement proteins[23].

The dose-dependent decrease in the mean difference of immunological paw edema indicates immunosuppression. The suppression may be attributed to the suppression of cellular immunity via the suppression of TH1 T lymphocyte pathway. Thus, leading to a decrease in the production and release of interleukin-12 (IL-12), the cytokine that favors TH1-mediated immune response and regulate the level of lymphocyte and macrophage needed to produce erythema, edema leading to severe dermal inflammation[24-25]. The immunosuppression exhibited by ARF on cell mediated immunity indicates an anti-inflammatory activity and may be regarded as being immune-protective and could play a vital role in defense against infectious organisms and infection of the foreign graft[26].

Haemagglutination is a primary parameter for the study of humoral response that involves antigen and antibody reactions. Humoral immunity denotes primary and secondary

immune responses to antigens that are mediated by antibodies. The significant ($p<0.05$) increase in the humoral antibody titer in the groups administered 50, 100 and 200 mg kg^{-1} b.wt., ARF relative to the normal control in both the primary and secondary immune response could be attributed to the immune-stimulatory activity of the ARF. The observed increase in the primary and secondary titers may also be attributed to the activation of the TH2 helper cells pathway which favors formation of interleukin 4 (IL-4) and secretion of lymphokine by TH2 cells that stimulate class-switching in B cells and promote IgE antibodies synthesis[27]. The increase in secondary responses associated with the significant ($p<0.05$) increase in secondary antibody titer, are due to the stimulation of the memory B cells which could result in high level of protection and could induce life-long immunity following infection[26].

Carbon tetrachloride induced hepatic injury is often used as a model for hepatoprotective drug screening and the extent of the hepatic damage is assessed by the level of cytoplasmic transaminases (ALT and AST) in circulation[28]. The present study revealed a significant increase ($p<0.05$) in the activities of ALT and AST in serum when a low dose of alkaloid rich fraction of the *Ricinus communis* seeds was administered to rats with CCl_4 induced liver damage and moderate effect on ALP indicating considerable hepatocellular injury. It increased ALT and AST levels in the test groups relative to that of the negative control but when the test groups were treated with low dose of the alkaloid rich fraction of *Ricinus communis* seeds, the ALT and AST levels were found to be significantly restored relative to the positive control. The reversal of increased serum enzymes in the CCl_4 induced rats when treated with doses of alkaloid rich fraction by attributed to its ability to prevent of the leakage of intracellular enzymes through membrane stabilization[29]. However, significant increase in ALT and AST activities were observed on the administration of high dose of the fraction when compared with that of positive and negative control indicating further injuries to the liver. Thus, at higher concentration, the alkaloid-rich fraction of *Ricinus communis* lacks hepatoprotective effect. It has been reported earlier that fresh leaves and aqueous extract of *Ricinus communis* offered protection against CCl_4-induced hepatic damage in albino rats[30]. This hepatoprotective effect was observed among the test groups that received low doses (100 mg kg^{-1} b.wt.) of the alkaloid rich fraction of the RC seed extract but at higher doses, the effect was contrary.

CONCLUSION

The result of this study suggests that the alkaloid rich fraction possesses anti-inflammatory activity, potentials for stimulating immune response and hepatoprotective effects against CCl_4 induced liver damage in male Wistar albino rats and possible against other compounds with similar effects on liver integrity and functions at higher doses.

SIGNIFICANCE STATEMENTS

The study discovers the possible synergistic effect of the alkaloid rich fraction of *Ricinus communis* seeds in improving immune system through its stimulatory effects and increased phagocytic of polymorphonuclear neutrophils. The alkaloid rich fraction possesses hepatoprotective and anti-inflammatory activities. This study will help the researcher to uncover the critical area of immunosuppression and hepatotoxicity that many researchers were not able to explore. Thus, new theories on the use of alkaloid rich fraction of *Ricinus communis* seeds and possibly its individual alkaloid components on the prevention of hepatotoxicity, immunosuppression and inflammation may be discovered.

REFERENCES

1. De Assis Junior, E.M., I.M. dos Santos Fernandes, C.S. Santos, L.X. de Mesquita, R.A. Pereira, P.B. Maracaja and B. Soto-Blanco, 2011. Toxicity of castor bean (*Ricinus communis*) pollen to honeybees. Agric. Ecosyst. Environ., 141: 221-223.
2. Rakesh, M.R., M.P. Kabra and V.S. Rajkumar, 2011. Evaluation of antiulcer activity of castor oil in rats. Int. J. Res. Ayurveda Pharm., 2: 1349-1353.
3. Ilavarasan, R., M. Mallika and S. Venkataraman, 2006. Anti-inflammatory and free radical scavenging activity of *Ricinus communis* root extract. J. Ethnopharmacol., 103: 478-480.
4. Singh, R.K., M.K. Gupta, A.K. Singh and S. Kumar, 2010. Pharmacognostical investigation of *Ricinus communis* stem. Int. J. Pharm. Sci. Res., 1: 89-94.
5. Taur, D.J. and R.Y. Patil, 2011. Antiasthmatic activity of *Ricinus communis* L. roots. Asian Pacific J. Trop. Biomed., 1: S13-S16.
6. Kensa, V.M. and Y.S. Syhed, 2011. Phytochemical screening and antibacterial activity on *Ricinus communis* L. Plant Sci. Feed, 1: 167-173.
7. Rana, M., H. Dhamija, B. Prashar and S. Sharma, 2012. *Ricinus communis* L.-A review. Int. J. PharmTech Res., 4: 1706-1711.
8. Panghal, M., V. Kaushal and J.P. Yadav, 2011. *In vitro* antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Ann. Clin. Microbiol. Antimicrobials, Vol. 10. 10.1186/1476-0711-10-21.

9. Rahmati, H., S. Salehi, A. Malekpour and F. Farhangi, 2015. Antimicrobial activity of castor oil plant (*Ricinus communis*) seeds extract against gram positive bacteria, gram negative bacteria and yeast. Int. J. Mol. Med. Adv. Sci., 11: 9-12.
10. Okwesili, F.C.N., C.N.A. Paul, E.J. Parker and U. Robert, 2016. Phytochemical, antihyperglycemic and lipid profile effects of methanol extract fraction of *Ricinus communis* seeds in alloxan induced diabetic male wistar albino. Asian J. Biochem., 11: 24-33.
11. Parker, E.J., P.C. Nweje-Anyalowu, O.F.C. Nwodo and R.I. Uroko, 2015. Effect of methanol extract of *Ricinus communis* (RC) seeds on blood glucose levels, antioxidant enzymes and haematological parameters of alloxan induced male Wistar Albino rats. Res. J. Med. Plant., 10: 140-149.
12. Ismail, S. and M. Asad, 2009. Immunomodulatory activity of *Acacia catechu*. Indian J. Physiol. Pharmacol., 53: 25-33.
13. Gifford, R.H. and S.E. Malawista, 1970. A simple rapid micromethod for detecting chronic granulomatous disease of childhood. J. Lab. Clin. Med., 5: 511-519.
14. Ganachari, M.S., S. Kumar and K.G. Bhat, 2004. Effect of *Ziziphus jujuba* leaves extract on phagocytosis by human neutrophils. J. Nat. Remedies, 4: 47-51.
15. Sharma, M.L., B. Singh, B.K. Chandan, A. Khajuria and A. Kaul *et al*., 1996. Actions of some flavonoids on specific and non-specific immune mechanisms. Phytomedicine, 3: 191-195.
16. Garbacki, N., V. Gloaguen, J. Damas, P. Bodart, M. Tits and L. Angenot, 1999. Anti-inflammatory and immunological effects of *Centaurea cyanus* flower-heads. J. Ethnopharmacol., 68: 235-241.
17. Nelson, D.A. and P. Mildenhall, 1967. Studies on cytophilic antibodies. 1. The production by mice of macrophage cytophilic antibodies to sheep erythrocytes: Relationship to the production of other antibodies and the development of delayed-type hypersensitivity. Aust. J. Exp. Biol. Med. Sci., 45: 113-120.
18. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
19. IBM., 2011. IBM SPSS Statistics for Windows, Version 20.0. IBM Corp., Armonk, New York, USA.
20. Joshi, T., J.P. Butchar and S. Tridandapani, 2006. Fcγ receptor signaling in phagocytes. Int. J. Hematol., 84: 210-216.
21. Njoku, U.O. and O.F.C. Nwodo, 2015. Immunomodulatory potentials of alkaloid rich fraction of *Abrus precatorius* seed methanol extract. Wulf. J., 22: 182-190.
22. Kumar, A., V. Singh and S. Ghosh, 2011. An experimental evaluation of *in vitro* immunomodulatory activity of isolated compound of *Ricinus communis* on human neutrophils. Int. J. Green Pharm., 5: 201-204.
23. Janeway, P.A., P. Travers and M. Wlport, 2001. The complement system and innate immune system. J. Immunobiol., 162: 412-450.
24. Ghani, S., M. Feuerer, C. Doebis, U. Lauer and C. Loddenkemper *et al*., 2009. T cells as pioneers: Antigen specific T cells condition inflamed sites for high rate antigen-non-specific effector cell recruitment. Immunology, 128: e870-e880.
25. Shourie, A. and K. Kalra, 2013. Analysis of phytochemical constituents and Pharmacological properties of *Abrus precatorius* L. Int. J. Pharm. Bio. Sci., 4: 91-101.
26. Tilwari, A., N.P. Shukla and U.D. Pathirissery, 2011. Immunomodulatory activity of the aqueous extract of seeds of *Abrus precatorius* Linn (Jequirity) in mice. Iran. J. Immunol., 8: 96-103.
27. Yadav, S.S., Galib, P.K. Prajapati, B.K. Ashok and B. Ravishankar, 2011. Evaluation of immunomodulatory activity of *Shirishavaleha*: An Ayurvedic compound formulation in albino rats. J. Ayurveda Integr. Med., 2: 192-196.
28. Janbaz, K.H., S.A. Saeed and A.H. Gilani, 2002. Protective effect of rutin on paracetamol- and CCl_4-induced hepatotoxicity in rodents. Fitoterapia, 73: 557-563.
29. Yaqub, H., N.A. Abdel Baky, L. Faddah and H.A. Attia, 2008. Hepatoprotective effect of N-acetyl cysteine and/or β-carotene on monosodium glutamate-induced toxicity in rats. Res. J. Med. Med. Sci., 3: 206-215.
30. Sabina, E.P., M. Rasoola, L. Mathew and Parameswarib, 2009. Studies on the protective effect of *Ricinus communis* leaves extract on carbon tetrachloride hepatotoxicity in albino rats. Pharmacologyonline, 2: 905-916.