Paracetamol-Induced Liver Damage and Effect of *Prosopis africana* Seeds Extract on Liver Marker Enzymes of Wistar Albino Rats

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Abstract. This study was carried to determine the effect of *Prosopis africana* seed extract after induction of paracetamol in Wistar albino rats to ascertain its ameliorating capacities in terms of liver damage. The assay was done on alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and hematological parameters. Thirty-two Wistar albino rats with an average weight of 120-180 g were used for this investigation. The *P. africana* seeds were extracted by maceration after 24 h fermentation in chloroform-methanol ratio 2:1. The animals were administered 2500 mg/kg of paracetamol upon 24 h starvation to induce liver damage. After administering 5000 mg/kg body weight (BW), the extract showed no acute toxicity. Serum alanine phosphatase, alanine aminotransferase, and aspartate aminotransferase levels decreased in all the test groups as compared to paracetamol group. The results depicted that *P. africana* seeds extract are effective in the regeneration of the damaged liver cells by averting enzyme leakage into the bloodstream.

Keywords: paracetamol, *Prosopis africana*, liver marker, enzymes, diabetes

Introduction

Researchers have found the use of medicinal plants as mujor source of treating disease such as *Garcinia kola*, with tremendous benefit for treating asthma, *Carica papaya* to cure hypertension, *Ocimum basilicum* a medicament for typhoid fever and *Cola nitida* for treating pile (FAO, 1996). In some West African countries such as Nigeria, fermented *Prosopis africana* seeds are used as a food seasoning. According to Kolapo *et al*. (2009), the stem and roots of *P. africana* have the potential for dentifrices production. The bark of *P. africana* was used by Lawal *et al*. (2010) to study its effect on the pile. The anticonvulsant activities of *P. africana* were observed after inducing convulsion with STR (strychnine) and PTZ (pentylenetetrazol). The plant extract was able to resist the effect of PTZ and STR induced convulsions (Ngo-Bum *et al*., 2009). The *P. africana* plant extract was checked for its anti-trypanosomal activity (Osho and Lajide, 2012). They reported the plant's bioactive ingredients which may help with the production of trypanocide that is potent and less toxic than other trypanocidal drugs. The *P. africana* is in the family of Fabaceae, it employs locally in phytomedicine in most African nations. This includes their uses for treating headache and toothache; their leaves and stem bark are mixed together in

managing rheumatism, the roots are applied for treating diuretic and dysentery, bronchitis, and stomach cramps (Arbonnier, 2002: Gilbert and Neil, 1986). Prosopis liquid is used as bio-adhesive agent or emulsifier to deliver metformin a therapy for diabetes. This illustrated the diverse application of the plants (Momoh *et al*., 2008; Adikwu and Nnamani, 2005).

Prosopis africana leafs extracts have hepatoprotective properties (Ojo *et al*., 2006), this was associated with the high tannin level in the leaves. *P. africana* is a leguminous plant; it is a flowering plant named differentially as "kiriya" in Hausa, "open" in Ibo and Idoma and "gbaaye" in Tiv languages of Nigeria. The leaves, branches, bark, and roots are used for different purposes in local medicines (Kalinganire *et al*., 2007). The tree is about 12 -18m tall; the dry pods have 10-15cm length and about 2cm in thickness (Ogunshe *et al*., 2007).The seed is frequently used in Nigeria as seasoning and formulation of animal feed (Aremu *et al*., 2006). Thus, this study was designed to check the effect of fermented *P. Africana* seeds extract with methanol, after inducing paracetamol to cause liver damage in Wistar albino rats.

Materials and Methods

Collection of plant material. The seeds of *P. africana* were obtained from Ogige Market – Nsukka - Nigeria

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and fermented for twenty-four hours in chloroform-methanol.

Preparation of paracetamol (Acetaminophen). The purchased paracetamol was the product of Emzor pharmaceutics, five tablets of 500 g each were ground and 2500 mg/kg BW was suspended in normal saline before oral administration, following Mitchell *et al*. (1973) method.

Extraction of seeds. The extraction was performed in line with Folch *et al*. (1957) method.

Experimental design. Thirty-two albino rats with weight-120-180 g were applied during this investigation. The subjects were housed and fed with water *ad libitum* for seven days prior to the experiment. After acclimatization, eight groups of the animals were separated with four rats per group. The administration of the extracts was made by oral intubation tube. The groups and doses administered are summarised in groups as outline stepwise.

  Group one: received 5 mL/kg of normal saline for 14 days (Normal rats and control).
  Group two: received 2,500 mg/kg of paracetamol suspension on day 7 and day 14 (Positive control).
  Group three: received 100 mg/kg BW of extract for 14 days.
  Group four: received 400 mg/kg BW of extract for 14 days.
  Group five: received 100 mg/kg BW of extract + 2500 mg/kg of paracetamol for seven days. Note that paracetamol was administered after 30 mins of administrating extract on the 7th day, while the extract administration on this group proceeded for the next seven days.
  Group six: received 400 mg/kg BW of extract + paracetamol 2500 mg/kg. (The same as group 5)
  Group seven: received 2500 mg/kg paracetamol + 100mg/kg BW of extract. Paracetamol was given on the 1st day while treatment with the extract commenced after 24 h for 14 days.
  Group eight: received 2500 mg/kg paracetamol + 400mg/kg bw of extract. (The same as group 7) Groups five and six represent the ameliorating effects of the extract on the liver marker enzymes while groups 7 and 8 represent the curative effects of the extract.

Blood was collected into sample bottles via retrobulbar plexus in the eye and mixed with 10% w/v anticoagulant in distilled water to prevent clotting. The assay for serum biochemical and hematological parameters was collected and analyzed intermittently on the 7th and 14th day.

Acute toxicity analysis of *P. africana* seeds extracted with methanol. The Lorke (1983) method was employed to determine the short (acute) toxicity of the *P. africana* seeds extracted with methanol in eighteen Wistar albino rats.

Induction of liver damage. Mitchell *et al*. (1973) method was used for the induction of liver damage in rats. Dacie and Lewis (1991) technique were employed to assay the hematological parameters of rats treated with *P. africana* seeds, extracted with methanol.

The assay of packed cell volume (PCV). PCV was assayed with microhaematocrit technique, where a mixed whole blood was permitted to enter capillary hematocrit duct together with anticoagulant until they are close to - 2/3 filled with blood. Blood was filled inside a plastic – sealed tube and placed in the medial groves. All tubes were spun for five minutes at 1000 rpm and calculated using equation 1.

$$PCS\ (\%) = \frac{\text{Height of cell column}}{\text{Height of total blood column}} \times 100$$

where: PVC = pack cell volume

The assay of HB (hemoglobin) concentration. Whenever a whole blood is added into Drabkin's reagent, a solution - KCN and kite $(CN)_6$, KCN will convert Hb-Fe^{2+} (ferrous) into Hb-Fe^{3+} (ferric), resulting in the formation of methaemoglobin which later combines with KCN to produce a stable pigment-cyanmethaemoglobin complex. The intensity of this colour mixture is measured in a spectrophotometer at 540 nm.Optical density (OD) of the solution will be proportional to the hemoglobin concentration. All forms of Hb - Hb-C, and Hb-O, except Hb-S, are determined by cyanate-technique. A 5.0 mL of Drabkin's reagent was pipetted into two test tubes 1 and 2 and a thoroughly mixed sample of EDTA treated blood (0.02 mL) was pipetted into the tubes, rinsing the pipette five times with the reagent, until all the blood was removed from the pipette. The solutions allowed standing at 25 °C for 10 min in order to allow the formation of cyano-methaemoglobin. The mixtures were read with a spectrophotometer at 540 nm by setting the percentage transmittance at 100%. The Drabkin's

reagent in tube 1 was used as a blank. The results were recorded using a pre-calibrated chart while Hb values in g/dL were determined.

The assay of white blood cells (WBCs) counts. Whenever whole blood is added to a solution of weak acids, their dilutions will haemolyse the red blood cells and enable the counting of white blood cells.

Filling counting chamber. Approximately 0.02 mL of well-mixed EDTA-anticoagulated blood sample was added to 0.38 mL of diluted liquid and dispensed into a small container. One of the grids of the counting chamber was filled with re-mix of the diluted blood sample using a Pasteur pipette. The filled area was left 2 min to allow time for the white blood cells to settle, after which the underside of the chamber was dried and viewed with a microscope.

Counting of white blood cells. Using the x10 objective with the condenser iris closed considerably to give good contrast, the ruling of the chamber and white cells were focused until the cells appeared as small black spots. The cells which are in the four extended squares of the chamber were counted squarely and calculated.

The counting and assay of red blood cell (RBCs). Principle: Whenever whole blood is diluted with isotonic liquid, it averts lysis and promotes the counting of RBCs. Also, some employed isotonic solutions include Hayem's solution and 0.85% sodium chloride (NaCl) solutions.

By adopting Thoma (manual) counting approach, anti-coagulated 0.5 mL of blood was pipetted and diluted to a 101mL with RBC diluting fluid (1: 200 dilution). Dilution was repeated in replicate. The chamber was filled by first expelling drops of the mixture and allowed for approximately 3 min. The red cells were counted and calculated using equation 2

$$RBC = CC \times CV \times CD$$

where:

RBC is red blood cells (in mm^3)
CC is cells counted
CV is a correction for volume
CD is a correction for dilution
Number of RBCs counted in five squares $\times 10^4$.

Determination of aspartate aminotransferase (AST). For determination of AST, alanine aminotransferase (ALT), alkaline phosphatase (ALP) methods of Klein *et al*. (1960) and Reitman and Frankel (1957) were used. Modified method by Shinde *et al*. (1999) was used to determine the effect of the extract on membrane stabilization, by evaluating the hypo tonicity-induced haemolysis of human red blood cells.

Statistical analysis. The T-test and one-way analysis of variance was applied to compare between groups and significance level was accepted at p < 0.05.Data are presented in mean ± standard deviation.

Results and Discussion

Paracetamol is a known antipyretic and analgesic drug which can cause liver necrosis if taken in an increased dosage. It eliminates sulphate and glucuronide conjugate (Mitchell *et al*., 1973). Taking paracetamol at high doses may cause the sulphation and glucuronidation pathway to attain saturation state. Thus, at a higher dosage of paracetamol, cytochrome-P 450 converts it into a highly reactive N-acetyl-*p*-benzoquinoline. The inhibitor of CYPs curtails the toxicity of paracetamol (Tanaka and Misawa, 2000). A radical of semiquinone gotten from one electron can cause the deregulation of N-acetyl-*p*-benzoquinoline, by covalently binding to cellular membrane macromolecules, which causes elevation of lipid peroxidation and tissue damage. At a higher dosage of paracetamol and N-acetyl-*p*-benzoquinoline may alkylate by oxidizing the intracellular GSH which reason the deregulation of liver GSH pool, proceeding to increase lipid peroxidation; thereby potentiating liver damage (Arnaiz *et al*., 1995). The choice of *P. africana* was based on its pharmacological properties as reported by Arbonnier (2002). In this study, the potential effect of *P. africana* seeds on - alkaline phosphatase, alanine aminotransferase, aminotransferase aspartate and other hematological parameters were investigated that are indicators of liver damage . The LD_{50} of the extract of *P. africana* did not show toxic effects at 5000 mg/kg b.w., suggesting the safety level of the seed extract if used at a high dosage (Table 1). The high degree of safety is consistent with the popular use of the fermented seed as food condiment to enhance the flavors of foods (Achi, 2005).

The packed cell volume, and hemoglobin level of the experimental animals in groups 5, 6 and 7 was treated with 100 mg/kg BW of paracetamol, and later treated with 400 mg/kg BW of the extract (Fig. 1). PCV expressed the packed red blood cells (RBC) in percentage

Table 1. Median lethal dose of *P. africana* seeds extract.

| Group	Dose (mg/kg) BW	Mortality
Phase one		
Group one	10	0/3
Group two	100	0/3
Group three	1000	0/3
Phase two		
Group one	1600	0/3
Group two	2900	0/3
Group three	5000	0/3

by volume of a whole blood sample after centrifugation (Wynne and Edwards, 2003).

The hemoglobin (Hb) assay ascertained the volume of Hb g/ dL of whole blood and quantified the oxygen-carrying ability of the red blood cells. The increase in PCV and hemoglobin concentration may suggest that the rats were not anemic and the oxygen-carrying capacity of the animals was not compromised. A significant ($p < 0.05$) decrease in the PCV of group 2 (paracetamol group) compared with the group 1 (control group) suggests that the animals were anemic due to

damage caused to the liver via paracetamol-induction (Fig. 2).

A significant ($p<0.05$) increase was recorded for total white blood cell of groups 3, 4, 5 and 6 rats, respectively, compared to group 2 (paracetamol group). WBC represents the cubic millimeter of whole blood and usually beneficial in defending the body to avoid infections (Schalm *et al.*, 1975). The increase in WBC count could be a result of the response to the induced liver damage that lead to the mobilization of the white cells. Though WBC counts increase rapidly, following an external attack by exogenous factors in response to boosting the body defense mechanism (Eyong *et al.*, 2004). This explains the cause for the increased WBC count (Fig. 3). The significant ($p < 0.05$) decrease in group 2 (paracetamol group) compared to the control group suggests suppression in WBC production due to paracetamol induction.

The elevation in RBC counts of the test groups was recorded, though the elevation was not significant ($p > 0.05$) relative to group 2 (paracetamol group), except for group 3 administered 100 mg/kg BW of extracts
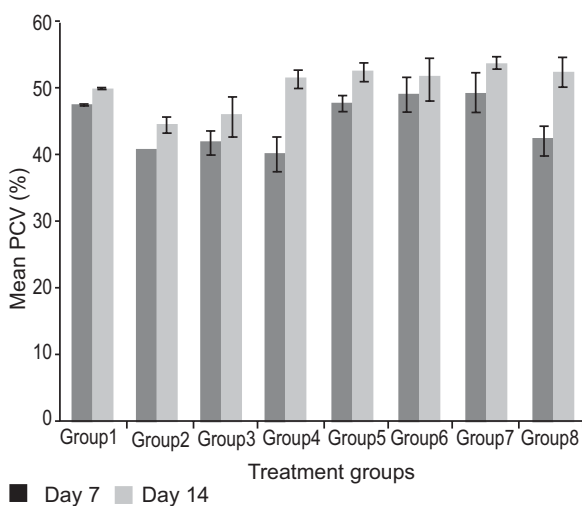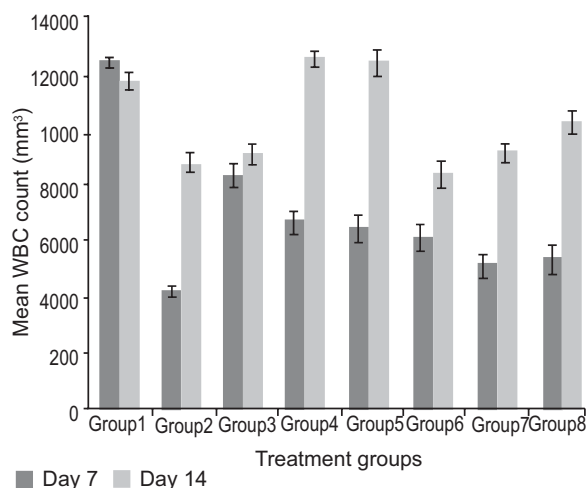

Group one = Normal control; Group two = Paracetamol (2500mg/kg BW); Group three = 100mg/kg BW of extract; Group four = 400mg/kg BW of extract; Group five = 100mg/kg BW of extract + paracetamol; Group six = 400mg/kg BW of extract + paracetamol; Group seven = paracetamol + 100mg/kg BW of extract; Group eight = PCM + 400mg/kg BW of extract;

Fig. 1. Effect of *Prosopis africana* seed extract on packed cell volume after paracetamol-induction of liver damage in rats
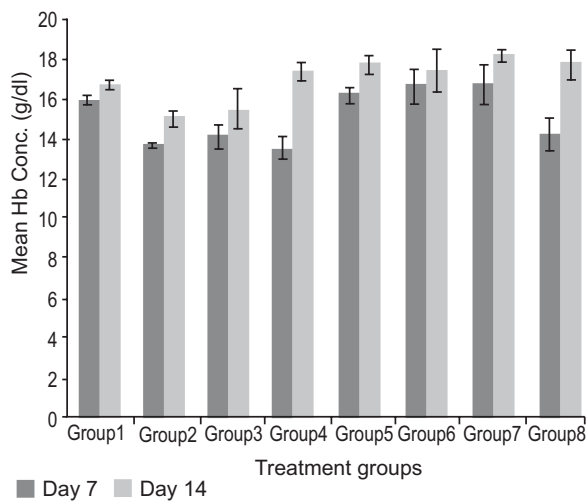
Group one = Normal control; Group two = Paracetamol (2500mg/kg BW); Group three = 100mg/kg BW - extract; Group four = 400mg/kg BW - extract; Group five = 100mg/kg BW - extract + Paracetamol; Group six =400mg/kg BW - extract + paracetamol; Group seven = Paracetamol + 100mg/kg BW - extract; Group eight = Paracetamol + 400mg/kg BW - extract;

Fig. 2. Effect of *Prosopis africana* seeds extract on haemoglobin concentration in paracetamol-induced liver damage of rats
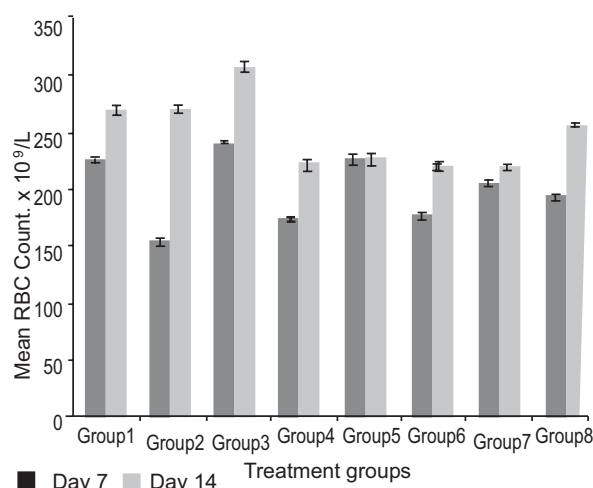
Group one = Normal control; Group two = Paracetamol (2500mg/kg BW); Group three = 100mg/kg BW - Extract; Group four = 400mg/kg BW - Extract; Group five = 100mg/kg BW of Extract + Paracetamol; Group six = 400mg/kg BW of Extract + paracetamol; Group seven = Paracetamol + 100mg/kg BW – Extract; Group eight = Paracetamol + 400mg/kg BW - extract;

Fig. 3. Effect of methanol seed extract of *Prosopis africana* on total white blood cell count in paracetamol-induced liver damage in rats

Group one = Normal control; Group two = Paracetamol (2500 mg/kg BW); Group three = 100mg/kg BW - Extract; Group four = Extract - 400mg/kg BW; Group five = 100mg/kg BW - Extract + Paracetamol; Group six = 400mg/kg BW of Extract + Paracetamol; Group seven = Paracetamol + 100mg/kg BW - Extract; Group eight = Paracetamol + 400mg/kg BW - extract

Fig. 4. Effect of methanol seed extract of *Prosopis africana* on red blood cell count in paracetamol-induced liver damage in rats

only. The main role of RBC is to transport oxygen to all organs, including lungs as well as transport carbon (IV) oxide from the cells to the lungs (Nelson and Cox, 2000). It was conceived that the extract may contain some mineral such as iron that helps to boost the iron level to transport oxygen to the cells. A decrease observed in group 2 (paracetamol group) as compared to normal control (group 1) was attributed to the RBCs destruction in the spleen (hypersplenism), that acquired alterations in the red cell membrane of group 2 rats induced with paracetamol (Fig. 4).

The assay of enzyme activities such as ALT and AST for assessment of paracetamol-induced liver damage or any other hepatotoxin are frequently used (Dobbs *et al*., 2003). Enzymes are released into circulation due to necrosis or membrane damage, thus, it is measured in serum or whole blood. In the mitochondria-hepatocytes, AST and ALT are predominantly localized in the liver. Therefore, it is an appropriate marker to check for liver impairment or insufficiency. Serum ALP is also associated with liver cell damage. Increase in the activity of these enzymes would suggest liver impairment. Administration of paracetamol caused a considerable increase of AST, ALT and ALP activities,

exemplified in Fig. 5-7, respectively. The damage caused to the conformation of the liver was due to the permeability of paracetamol into the cytoplasmic domain which provoked the release of these enzymes into the bloodstream after cellular damages, which suggest the onset of hepatotoxicity (Sallie *et al*., 1991).

There was a significant ($p < 0.05$) elevation in the activities of ALT and AST of group 2 rats compared with the rats in normal control (group 1). The result of this study also shows a reasonable reduction in the AST level of groups 6, 7 and 8 rats treated with 400 mg/kg BW of extract after 100 mg/kg BW paracetamol induction. The 400 mg/kg BW of the extract was administered after paracetamol induction, it was compared with group 2 (paracetamol group) to establish the variation in the effect of paracetamol. The result deduced that at a higher dose - 400 mg/kg BW of the extract, the injurious effects of paracetamol were overcome. Furthermore, the 100 and 400 mg/kg BW of the extract administered after paracetamol induction was able to ameliorate the damage to an extent. Significant ($p < 0.05$) reduction was recorded in the ALT activity of all the test groups compared with group 2 (paracetamol group). This result actually indicates

the protective and ameliorative effect of the extract since ALT is more localized in the liver. Thus it is a better marker of effect for estimating liver injury. Its protective activity indicates that hepatic tissue damage was repaired and plasma membrane stabilized. This could be attributed to flavonoids and alkaloids in the extract. The lower and normal (ALT and AST) activities suggest vitamin B6 deficiency. The results of AST and ALT agreed with an earlier report by Ramadoss *et al*. (2011) and Ojo *et al*. (2006). The liver injury was caused by the toxicity of paracetamol, which caused an increase in the levels of serum alkaline phosphatase (ALP) (Rajesh and Latha, 2004). ALP elevation in group 2 rats compared with the normal group could be associated with cholestasis due to biliary obstruction or hepatic infiltration of rats in group 2. Day 7 shows a non-significant ($p > 0.05$) increase of ALP activity in all the test groups when compared with the control groups (group 1 and 2), while day 14 shows a non-significant ($p > 0.05$) decrease in groups 4, 5, 6, 7 and 8 rats, compared groups 1 and 2. This result suggests that the extract was able to regenerate the damaged hepatic cells with time. Furthermore, ALP activity of group 3 rat's administered 100 mg/kg BW of extract only was found to be elevated as compared with the control groups throughout the experiment. This result implies that the extract was not able to control the activity of ALP. It was reported that effective control of alkaline phosphatase activity is associated with the early improvement and repair of the mechanism of secreting the hepatic cells (Mason, 2004).

The extract was effective in ameliorating paracetamol effect on live damage in this study. These parameters were repaired or reinvigorated - plasma membrane stabilization, and repair of liver tissue damage. The result resorts that transaminase activities reversed to the normal healing process of the liver- parenchyma cells and the regeneration of liver tissues (Shukla *et al*., 1992; Thabrew *et al*., 1987). *P. africana* seed extract showed stabilization of membrane versus hypotonicity- induced hemolysis with the human red cells. The inhibition of induced-hemolysis is outlined in Table 2. The shrinkage of cells due to loss of osmotic intracellular electrolyte may cause hemolysis to arise. The processes that play a role in stimulating the efflux of intracellular components was inhibited by extract after administration. The induction of hemolysis via hypotonicity of red blood cells usually occurs because of uptake of water cells, which triggered the release of hemoglobin to absorb maximally at 418 nm. The hemolysis inhibition was dose-dependent, i.e. the response of the cell was increasing as the concentration of the extract increased. A dose-dependent decrease in the optical density shows a corresponding amount of hemoglobin released into the medium. Any toxicity usually results in distortion of liver membrane integrity due to hepatocellular damage and a rise in plasma levels r, a consequence of high toxin levels in hepatocytes. The stabilizing effect of this extract on lysosomal membranes suggests a possible action of anti-inflammation. This result corroborated the findings of Anosike and Obidua (2010) and Iwueke *et al*. (2006) who reported the stabilization of hepatocyte membrane with coconut extract and *Vitex doniana* leaves.

Conclusion

Following the result, one can infer that *Prosopis africana* seeds extracted with methanol have significant action on liver damage caused by paracetamol-induced hepatotoxicity. It is therefore, suggested that incorporation of *P. africana* seeds in the human diet may help prevent liver ambulation, ameliorate enzymes leakage and protect against liver damage.

Table 2. Effect of *Prosopis africana* seed extract on hypotonicity-induced hemolysis of human red blood cells

Treatment	Extract concentration (mg/mL)	Normal saline (mL)	Distilled water (mL)	RBC (blood) (mL)	Absorbance	% inhibition of haemolysis
Isotonic solution	-	1.8	-	0.2	0.051	-
Hypotonic solution	-	0.6	1.2	0.2	0.701	-
Test-sample	0.1	0.5	1.2	0.2	0.258	63.2
Test-sample	0.2	0.4	1.2	0.2	0.146	79.2
Test-sample	0.4	0.2	1.2	0.2	0.119	83.0
Test-sample	0.5	0.1	1.2	0.2	0.018	97.4

Conflict of Interest. The authors declare no conflict of interest.

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