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Research Article

EFFECT OF ARABICA AND CANEPHORA COFFEE BEAN EXTRACTS TOWARDS MODIFICATION OF RED BLOOD CELL SURFACE ANTIGENS

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ABSTRACT

Introduction: ABO and RhD variants are the most studied blood group in India. Progress is being made towards modification of red blood cell surface antigens from A and/or B to blood group O. Objective: The objective of this study is a comparative analysis of the changes observed on the red blood cell antigens which differ in a short glycoprotein chain difference, by the effects of a *Coffea arabica* and *Coffea canephora* bean extracts. Methods: The heamatological effect of the coffee bean extract was prepared and evaluated using blood group analysis on haematological indices. Results: Agglutination was clearly observed initially for 5 min for all the eight blood types. Specifically, for the O+ blood group there is a time lag for agglutination to take place for the incubation period of 15 min for *Coffea arabica*, while agglutination occurred after 7 minutes of adding blood group antibodies with *Coffea canephora*. Conclusion: These results, as well as the possibility of adapting this method to a fully automated system, could be an important contribution to the field of immunohematology.

KEYWORDS: *Coffea arabica*, *Coffea canephora*, RBC, Coffee bean, Glycoprotein, Agglutination

INTRODUCTION

Coffee bean extracts has been reported as antioxidant, anti-obesity and hepatoprotective activity[1]. In this study, coffee seed extracts showed stimulatory effect on the immune functions. Globally, blood is an essential part of modern healthcare. In the United States alone, nearly 5 million patients receive approximately 14 million units of red blood cells donated each year according to estimates by the American Association of Blood Banks and the National Blood Collection and Utilization Survey[2]. Morgan in UK and Iseki in Japan discovered that some bacterial enzymes from *Clostridium tertium, Clostridium welchii, Bacillus cereus* and *Trichomonas foetus* specifically destroyed A, B or H antigens[3]. Enzymatic conversion of type B blood using purified or recombinant coffee bean (*Coffea canephora*) α-galactosidase has been achieved using 100-200 U/ml[4]. Glycine max has been proposed to convert B cells in a more efficient way, but these protocols have not been completely evaluated by routine blood-typing tests or clinical trials[5]. The researchers homed in on two enzymes; of which one from a gut bacterium called *Bacteroides fragilis*, removes the B antigen[6]. WBC counts was also increased significantly ($p < 0.001$) in all doses of the plant extract. *Coffea arabica* extract elicited a significant ($p < 0.001$) increase in the DTH response at doses of 50 and 150 mg/Kg, but the change at higher dose of 250 mg/Kg was not statistically significant[7]. Many these plants and their isolated constituent have shown beneficial therapeutic effects, including antioxidant[8], anti-inflammatory[9], anticancer[10], antimicrobial[11], and immunomodulatory effects[12]. In present study, we have been undertaken to explore the effect of *Coffea arabica* and *Coffea canephora* coffee bean extract towards modification of red blood cell surface antigens.

MATERIAL AND METHODS

PREPARATION OF COFFEE BEAN SMOOTHIE

Extraction was carried out by grinding seeds of the plant with a pestle and mortar in the presence of liquid nitrogen and or made into smoothie[13]. The material was transferred to a vial and 500µl of lysis buffer (HiMedia) was added and the sample was vortexed. Later, the suspension was mixed for one hour at 37^{0}C and filtered. The filtrate was spin at 14,000 rpm (Thermo, MicroCL 21 Microcentrifuge) in cold conditions for 10 min and supernatant was removed and stored at 4^{0}C until further analysis.

BLOOD SAMPLE COLLECTION AND PROCESSING

Blood samples were obtained from eight healthy individuals (males and females) aged 18 to 20 years. Informed consent was obtained from everyone before collection of blood, and the procedures followed were in accordance with the ethical recommendations (23/IEC/IG/2017) of our institution[14]. The samples were kept at 4^{0}C and divided into groups, based on blood typing, using ABO diagnostic kit (Span Diagnostics).

CHEMICAL ANALYSIS OF EXTRACT

The alcoholic extract showed the presence of flavonoid, phenolics, glycoside, saponins, alkaloid, and polysaccharide when subjected to qualitative chemical tests. The total phenolic contents estimated were done according to standard published method Folin Ciocalteu reagent[15]. Aluminum chloride colorimetric method was used for flavonoid determination[16].

DETERMINATION OF BLOOD TYPE CONVERSION

Briefly, whole-blood cells from A, B, and O blood donated from healthy volunteers were loaded on agglutination plate. 5µL of the Anti-A or Anti-B or Anti-D antibodies were added to human A- or B-type whole-blood cells on a white acryl plate, and the agglutination reaction was determined within 30s of its onset[17].

RESULTS

BLOOD SAMPLE COLLECTION AND PROCESSING

All the samples collected from the informed consent have been processed and analyzed for its type. The blood was collected into a vacuum glass tube or heparin tubes used as anticoagulant for the determination of blood group. All eight samples were processed and analyzed using ABO diagnostic kit (Span) and represented in table 1.

Table 1: ABO blood typing of various samples

Sample ID	Age	Blood type
KLU01	19	A+
KLU02	19	A-
KLU03	20	B+
KLU04	19	B-
KLU05	20	AB+
KLU06	20	AB-
KLU07	19	O+
KLU08	20	O-

CHEMICAL ANALYSIS OF EXTRACT

Total phenolic and flavonoid contents were found about 13% and 3% respectively in the bean of *Coffea arabica* and *Coffea canephora*. Total phenolic content was determined according to Folin- Ciocalteau method. The concentration of phenolic compounds in test samples was calculated from the standard gallic acid curve. The standards graph of gallic acid and quercetin for total phenolic and flavonoid contents are shown in figures 1 and 2 respectively.

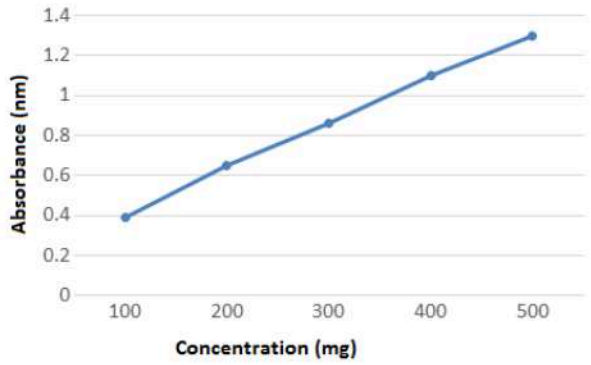


Figure 1: Standard curve of gallic acid for total phenolic contents

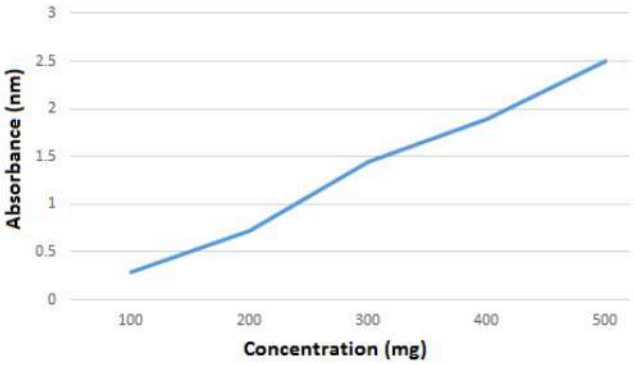


Figure 2: Standard curve of quercetin for total flavonoid contents

DETERMINATION OF BLOOD TYPE CONVERSION

Analysis was run in duplicates (n=2) to verify the difference in the effect of coffee bean extracts on blood samples. First, it is observed that there was no effect of different extracts of processing on the enzyme activities on blood group. Later for the increase in incubation time, the agglutination was gradually decreased. Specifically, for the O+ blood group there is a time lag for agglutination to take place for the incubation period for 15 min for *Coffea arabica*, while agglutination occurred after 7 minutes of adding blood group antibodies with *Coffea canephora* (Figure 3-10).

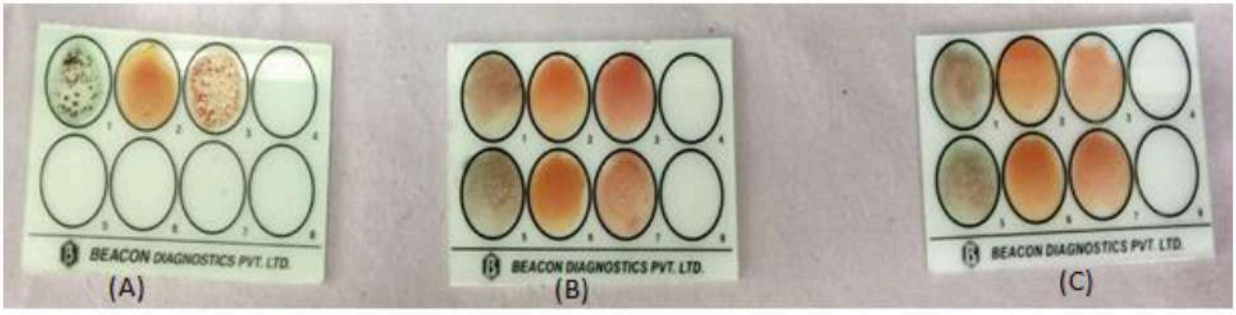


Figure 3: Hemagglutination assay of RBCs by ABO slide method for blood type A+. (A) Agglutination was observed in zone 1 and 3 indicating the test as positive for blood type A+ (positive control test) (B) Agglutination of A+ blood type was hampered after incubating with *Coffea arabica*, because zones were dark and uniform in color, indicating no agglutination. (C) Test is specific for *Coffea canephora* however, resulted in low level agglutination.

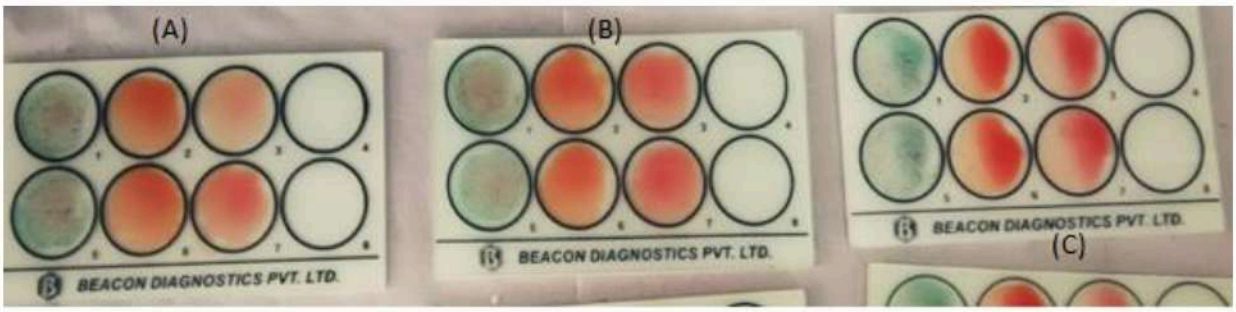


Figure 4: Hemagglutination assay of RBCs by ABO slide method for blood type A-. (A) Agglutination was observed in zone 1 indicating the test as positive for blood type A- (positive control test) (B) Test is specific for *Coffea arabica*, resulted in normal agglutination. (C) Test is specific for *Coffea canephora*, resulted in normal agglutination.

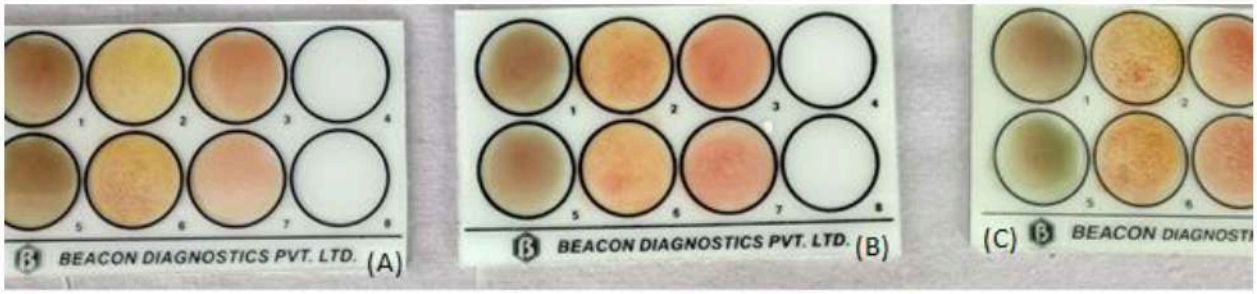


Figure 5: Hemagglutination assay of RBCs by ABO slide method for blood type B+. (A) Agglutination was observed in zone 2 and 3 indicating the test as positive for blood type B+ (positive control test) (B) Test is specific for *Coffea arabica*, resulted in normal agglutination. (C) Test is specific for *Coffea canephora*, resulted in high level agglutination.

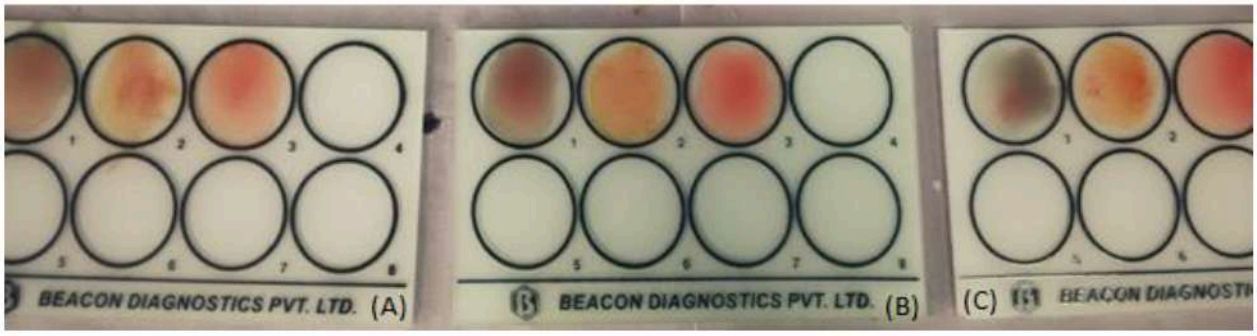


Figure 6: Hemagglutination assay of RBCs by ABO slide method for blood type B-. (A) Agglutination was observed in zone 2 indicating the test as positive for blood type B- (positive control test) (B) Test is specific for *Coffea arabica*, resulted in normal agglutination. (C) Test is specific for *Coffea canephora*, resulted in normal agglutination.

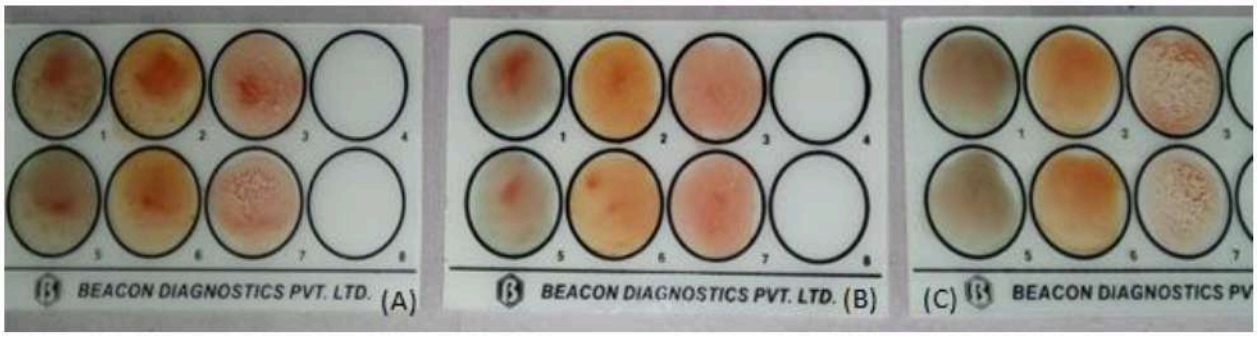


Figure 7: Hemagglutination assay of RBCs by ABO slide method for blood type O+. (A) Agglutination was observed in zone 3 indicating the test as positive for blood type O+ (positive control test) (B) Test is specific for *Coffea arabica*, resulted in low level agglutination. (C) Test is specific for *Coffea canephora*, resulted in high level agglutination.

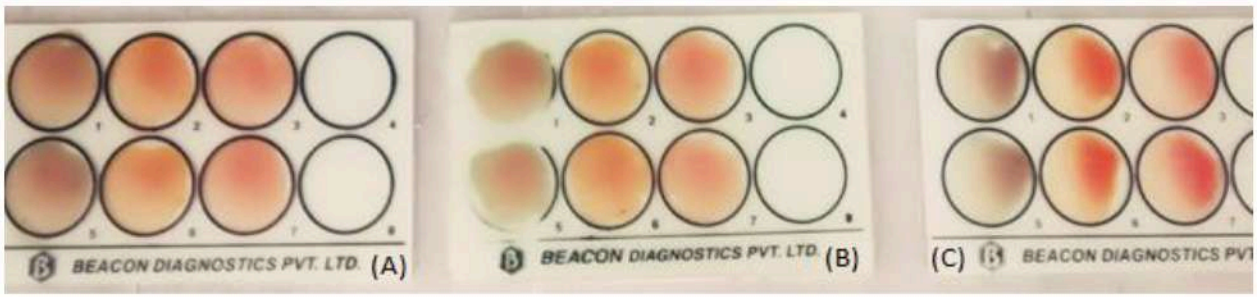


Figure 8: Hemagglutination assay of RBCs by ABO slide method for blood type O-. (A) Agglutination was not observed in either zone 1, 2 or3 indicating the test as positive for blood type O- (positive control test) (B) Test is specific for *Coffea arabica*, resulted in nil agglutination. (C) Test is specific for *Coffea canephora*, resulted in nil agglutination.



Figure 9: Hemagglutination assay of RBCs by ABO slide method for blood type AB+. (A) Agglutination was observed in zone 1, 2 and 3 indicating the test as positive for blood type AB+ (positive control test) (B) Test is specific for *Coffea arabica*, resulted in normal agglutination. (C) Test is specific for *Coffea canephora*, resulted in high level agglutination.

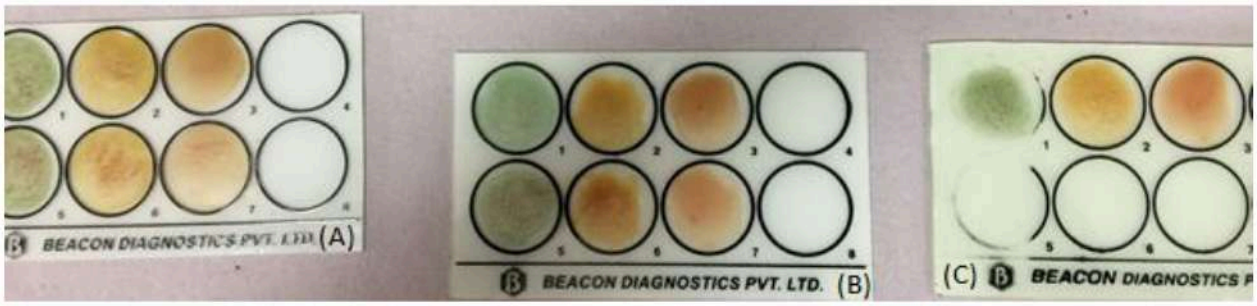


Figure 10: Hemagglutination assay of RBCs by ABO slide method for blood type AB-. (A) Agglutination was observed in zone 1, 2 and 3 indicating the test as positive for blood type AB- (positive control test) (B) Test is specific for *Coffea arabica*, resulted in nil agglutination. (C) Test is specific for *Coffea canephora*, resulted in nil agglutination.

DISCUSSION

The impact of coffee bean extracts on sample processing in the laboratory was evaluated for agglutination assays for ABO and D (Rh factor). For all the eight blood group samples agglutination initially for 30s was clearly seen for *Coffea arabica*[18] and *Coffea canephora*[19] at 0.05 mg/mL. Hemagglutination assay of RBCs by ABO slide method for all eight blood types were evaluated and found to be effective for blood type B+ compared to other types. Although further work has to be extensively carried out to determine the effect of the coffee bean extracts at high incubation periods and also for high concentration levels.

CONCLUSION

Traditional blood typing methods are simple, sensitive and reliable, yet time consuming and labor intensive; moreover, the cost of blood group-specific antibodies is quite high. Hence an immediate approach for the modification of red blood cell antigens is needed for a compatible blood transfusion. Here, we have described a comparative study for effect of coffee bean extracts for hemagglutination detection on all types of blood groups. The difference between the extracts were evaluated and estimated to determine the modification of RBC surface antigen. The agglutinated samples elicited changes between 10% and 35% in the O+ and B+, while the non-agglutinated samples induced a maximum change of 7.5% in all other six blood types.

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