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Carbohydrate fraction of *Aloe vera* ameliorates inflammation through suppression of pro-inflammatory mediators and oxidative stress *in vitro* and rats with Freund's adjuvant induced paw edema

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Aloe vera (L.) Burm. F (Asphodelaceae) is a traditional medicinal plant having multiple biological activities such as wound healing, anticancer and antidiabetic activity, etc. These properties have been attributed to the constituents present in *Aloe vera*. One of the major constituents is carbohydrate, which has not been explored much for its antioxidant and anti-inflammatory activity. Here, we evaluated the antioxidant and anti-inflammatory potential of carbohydrate fraction (CF) of *Aloe vera* extract using *in vitro* and *in vivo* model for its therapeutic application in the treatment of inflammation. CF extract was prepared using hot water extraction followed by ethanolic precipitation. The CF extract was tested for free radical scavenging assays, lipopolysaccharide-stimulated RAW264.7 cells *in vitro* and *in vivo* by Freund's adjuvant induced rat paw edema model. The free radical scavenging activity of CF extract significantly increased in a concentration-dependent manner with maximum inhibition observed at the concentration of 2 mg/mL. CF extract (20 and 60 µg/mL) showed their maximum cell viability in RAW264.7 cells by MTT assay. CF extract shows a considerable decrease of inflammatory mediators in both *in vitro* and as well as *in vivo* studies. The increase of superoxide dismutase and catalase levels in CF treated adjuvant induced rats decreases ROS/RNS levels and inflammatory mediators. It may be primarily activated by NF-κB and AP-1 indicating its potent antioxidant activity that promotes modulation in cellular redox state. The results suggest that carbohydrate fraction of *Aloe vera* could be useful for the management of oxidative stress and inflammatory diseases.

Keywords: *Aloe barbadensis*, Anti-inflammatory, Antioxidant, IL-6, Polysaccharides, TNF-α

Antioxidants act as a defense mechanism by scavenging free radicals which are formed during biochemical reactions in the biological system[1]. In oxidative stress, reactive oxygen species (ROS) play a principal role in the pathogenesis of inflammation[2]. Inflammatory response plays a major role in the host defense mechanism against stimuli like cell damage, allergens and foreign organisms[3]. Excessive inflammation leads to disorders, such as autoimmune diseases, cardiovascular disease, arthritis, etc. The progression of inflammation is characterized by immune cells such as macrophages and T cells, which infiltrate the tissue and synovial membrane of the joints affected with inflammation. This leads to accumulation of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), interleukin 1 beta (IL-1β), etc.[4]. The long term usage of available drugs leads to gastrointestinal bleeding, renal disorders and morbidity[5]. Due to these side effects and the high cost of available drugs, it has become prudent to look for plant based therapy to alleviate these symptoms.

Plant polysaccharides have multiple biological effects such as antioxidant activity[6], immunomodulatory activity, anti-inflammatory activity, antitumor, wound healing, antidiabetic activity, etc.[7]. It decreases ROS levels and regulates the inflammatory stimuli through various mechanisms such as decrease of free radicals formation and ROS pathway[8]. Polysaccharides conjugated with polyphenols also showed satisfactory antioxidant potential and metal chelating activity[9]. *Aloe vera* (L.) Burm. F (Syn. *Aloe barbadensis* Miller) is a traditional medicinal plant that belongs to the family Asphodelaceae (Liliaceae). *Aloe vera* has been reported to have various biological effects such as antioxidant, anti-inflammatory, antidiabetic, anticancer, antimicrobial, wound healing, neoplastic activity, etc.[10]. The medicinal properties have been attributed to the various phytoconstituents present in the *Aloe vera* such as carbohydrates, polyphenols, flavonoids, polypeptides or peptides, vitamins, sterols, enzymes and anthraquinones, etc.[11].

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Carbohydrates are one of the major bioactive constituents present in *Aloe vera* gel and they have been reported for various biological and therapeutic properties. Most of the studies have been reported either individually for evaluation of antioxidant[12] or anti-inflammatory activity[13] of *A. vera* polysaccharides. However, correlation of antioxidant and anti-inflammatory effects of *A. vera* polysaccharides has not been studied *in vitro* and *in vivo* models. Hence, we made an attempt to study both the antioxidant and anti-inflammatory potential of carbohydrate fraction of *Aloe vera* extract with respect to oxidative damage and inflammatory mediators through *in vitro* and adjuvant induced rat paw edema model.

Materials and Methods

Chemicals and drugs

All standard chemicals, TNF-α, IL-6ELISA kits and lipopolysaccharide, Complete-Freund's Adjuvant were procured from Sigma Aldrich (USA). Secretory Phospholipase A2 (sPLA2) ELISA kit was purchased from Cayman (USA). The RAW264.7 cell line was purchased from National Centre for Cell Sciences, (NCCS), Pune. Diclofenac and Aspirin drug were purchased from Cipla, India Ltd. All other chemicals used were of analytical grade purchased from Sisco Research Laboratories, Mumbai, India.

Plant collection, identification and preparation of carbohydrate enriched fraction (CF) from *Aloe vera* extract

Aloe vera leaves were collected from Chennai, Tamil Nadu, India and it was authenticated by the Botanical Survey of India, Southern Regional Centre, Coimbatore, India (BSI/SRC/5/23/2018/Tech/728). The carbohydrate enriched fraction was prepared by hot water extraction and cold ethanol precipitation with the modifcations[12]. *Aloe vera* extract was mixed in double-distilled water in the ratio of 1:20 (w/v) and boiled at 100±2°C for 4 h, centrifuged at 8000 rpm for 20 min, supernatant and pellet was collected separately. Further to the supernatant and pellet fractions obtained, 95% ethanol was added and kept at 4°C with stirring for precipitation for 5 days till the aqueous layer was clear. Both the pellets of CF of *Aloe vera* extract obtained from hot water extraction and cold ethanol precipitation were combined and freeze-dried in a Christ Alpha1-4-lyophilizer (Germany) and stored at 4°C till further use.

Analysis of monosaccharide composition of CF extract

The total carbohydrate content present in the CF extract was estimated by the phenol-sulfuric acid method using glucose as standard[14]. The monosaccharide composition was estimated by ion-exchange chromatography according to the modified method of Wang *et al.*[15]. The CF extract was hydrolyzed with 4M trifluoroacetic acid (TFA) at 121°C for 4 h in a sealed centrifuge tube and further hydrolyzed with 6N HCl for 1 h. The hydrolyzed sample was re-dissolved in double-distilled water and analyzed for monosaccharide composition with ICS 5000 SP Ion chromatography and a pulsed amperometric detector (Dionex, Sunnyvale, CA) with a CarboPac PA20 analytic column (150 ×x 3 mm inner diameter). The mobile phase consists of 300 mM NaOH (2%) and water (98%) at a flow rate of 0.3 mL/min.

Assessment of *in vitro* antioxidant and anti-inflammatory activities of CF extract

Antioxidant assays

Free radical scavenging activities of the CF extract at different concentrations (0.1, 0.25, 0.5, 1 and 2 mg/mL) were evaluated for ABTS, DPPH, H_2O_2 scavenging assays and metal chelating activity. ABTS and DPPH assays were performed as reported earlier[11,16,17]. Hydrogen peroxide assay was carried out as described by the modified method of Fernando *et al.*[18]. Metal chelating activity was measured as described by Saha *et al.*[19].

Percentage of free radical-scavenging and metal chelating activity was measured by the following equation: $[(A_c - A_s)/A_c] \times 100$, where A_c denotes the absorbance of the control reaction (containing all reagents except the sample) and A_s denotes the absorbance of the test sample.

Inhibition of albumin denaturation

Different concentrations of the CF extract (0.1, 0.25, 0.5, 1 and 2 mg/mL) were studied using of inhibition of bovine serum albumin (BSA) denaturation technique according to Chauhan *et al.*[20]. Aspirin was used as a reference drug. The percentage of inhibition in denaturation was measured by the following equation: 1-(D/C) × 100%, where D denotes the absorbance of the sample, C denotes the absorbance without test sample (negative control).

Membrane stabilization

CE extract of various concentrations (0.1, 0.25, 0.5, 1 and 2 mg/mL) was studied using red blood cells membrane stabilization method. Blood was collected from healthy male Wistar rats and a further method was followed according to Parameswari *et al.*[21]. Diclofenac was used as the reference drug.

Percentage protection =100 – (As/Ac) × 100

where Ac= absorbance of control, As = absorbance of test sample.

In vitro studies using RAW 264.7 cells

Murine macrophage RAW 264.7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 0.4% penicillin and streptomycin at 37°C in 5% CO_2 humidified atmosphere.

MTT cytotoxicity assay

The cytotoxicity assay was carried out by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. The RAW 264.7 cells at a concentration of 1×10^4 cells/well were inoculated in a 96-well microtiter plate at 37°C in 5% CO_2 for 12 h[22]. The cells were treated with different concentrations of CF extract (20, 40, 60, 80 and 100 µg/mL) and further incubated for 24 h. The cells were washed with 1X Phosphate Buffered Saline (PBS) and 20 µL of MTT solution (5 mg/mL) was added to each well. It was further incubated at 37°C for 4 h. MTT was removed by washing with 1XPBS and the formazan crystals were dissolved in 200 µL of DMSO. Optical density was read at 570 nm using DMSO as blank in a microplate reader (PerkinElmer, USA) and viability percentage was calculated. It was observed that 60 µg/mL of CF extract did not affect the cell viability. Two doses of CF extract (lower 20 µg/mL and higher 60 µg/mL) were selected for further following experiments.

Estimation of TNF-α and IL-6 levels in macrophage RAW264.7 cells

RAW 264.7 cells were seeded in a 24-well plate at a density of 2×10^4 cells/well overnight. Then the cells were pre-treated with the CF with 20 and 60 µg/mL concentrations for 2 h. The cells were further incubated with and without LPS added as an inflammatory stimulus (1.0 µg/mL, 20 µL) for 24 h. After incubation at 37°C overnight, media aspirated and centrifuged at 1500 rpm, 5 min, 20°C, the supernatant was collected and TNF-α, IL-6 was measured by the respective ELISA kits (Sigma Aldrich, USA), according to the manufacturer's instructions. Diclofenac (20µM, Cipla, India Ltd.) was used as the positive control[23].

Measurement of Nitric Oxide production

Nitric oxide production assay was performed according to Nauman *et al.*[24]. RAW 264.7 cells were seeded in 96-well plate at a density of 1×10^5 cells/mL per well and were treated with 20 and 60 µg/mL of the CF extract for 2 h, further stimulated with LPS (1.0 µg/mL) for 24 h at 37°C in 5% CO_2. Then 50 µL of cell media was harvested and mixed with 50 µL of Griess reagent. The mixture was incubated at room temperature (28±2°C) for 10 min and the absorbance was measured at 540 nm using fresh DMEM culture medium as a blank. The quantity of nitrite in the culture medium was measured as the amount of NO production and determined from a sodium nitrite standard curve.

Animals

Adult male healthy Wistar rats with a bodyweight ranging from 180 to 200 g were maintained in an air-conditioned room (25±2°C) with a 12 h light/dark cycle. The animals were acclimatized to the environment before the experimental use, by supplying water *ad libitum* and fed with a standard laboratory diet provided. All animals were treated and cared for as per following the guidelines recommended by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India. The animal experimental study was approved by the Institutional animal ethics committee (VIT/IAEC/12/July23/03/2016). The CF extract was freshly resuspended in 1.0 mL double distilled water and administered orally every day at a concentration of 54 mg/kg body wt. per animal, respectively. The dosage of CF of *Aloe vera* extract used in this study was selected based on our previous study[25] and diclofenac was used as the standard drug[26].

Experimental procedure

The rat paw edema assay protocol used was a slightly modified method of Mesharam *et al.*[27]. The animals were randomly allocated to four different groups comprising of six animals each as follows: Group I, Normal rats (N); Group II, Freund's adjuvant (0.1 mL) induced arthritic rats (IC); Group III, Freund's adjuvant induced arthritic rats treated with CF of *Aloe vera* extract (54 mg/kg body wt.) (IC+CF); and Group IV, Freund's adjuvant induced arthritic rats treated with diclofenac (10 mg/kg body wt.) (IC+DCF).

Complete-Freund's Adjuvant induced rat paw edema assay

Freund's adjuvant (0.1 mL heat-killed *Mycobacterium tuberculosis* (10 mg/mL) in paraffin oil) was injected intradermally into the footpad of the left hind paw of rat[22]. Treatment of Gr. III and IV Wistar rats were started after seven days of post-injection of administration of complete Freund's adjuvant and continued till day 14. Paw edema was assessed through physical and biochemical measurements. Paw

volume was assessed by the measurement of hind foot paw using the Vernier scale. The difference in paw volume on 1st and 14th day was considered as inflammatory edema. Volume changes in Gr. III and IV were compared with Gr. II. Percentage inhibition was calculated according to the method of Sarkhel[28]. The percentage of paw volumewais calculated as Percentage inhibition of edema = $(T\text{-}T_o/T) \times 100$ where T = Mean edema (thickness) of the inflammatory control group and T_o represent the Mean edema of treated groups, respectively. After the experimental period on day 15, the rats were sacrificed under mild ether anaesthesia. The blood was collected by cardiac puncture in the tube containing EDTA and centrifuged at 1800 rpm for 20 min at 4°C. Plasma was collected and used for estimation of TNF-α, IL-6, sPLA2 levels through respective ELISA kits.

Tissue homogenate

The liver and pancreas of rats were removed and washed with ice-cold PBS. Tissue homogenate was prepared in 50 mM phosphate buffer pH 7.4 at 4°C by ultrasonication for 3 min to give 10% tissue homogenate. The tissue homogenate was centrifuged at 4°C for 15 min at 8000 rpm. The supernatant was used for estimation of superoxide dismutase (SOD) and catalase activity.

Estimation of Superoxide dismutase activity

Superoxide dismutase activity was measured by the slightly modified method of Sharma *et al.*[29]. The assay was performed using 20 µL of tissue homogenate of liver and pancreas suspended in 2.8 mL of 50 mM Tris buffer pH 8.2. Pyrogallol was used as substrate at a concentration of 15 mM and 100 µL solution was added for each replicate. Absorbance was measured at 420 nm. The control solution contains100 µL of 50 mM Tris buffer with 2.8 mL of 15 mM of pyrogallol. The degree of inhibition of the auto-oxidation of pyrogallol at alkaline pH by SOD was used to measure the enzyme activity. SOD activity was expressed as 50% inhibition of auto-oxidation of pyrogallol. The specific activity was expressed as Units/mg of protein.

Estimation of catalase activity

Catalase activity was carried out as described by Sivaranjini *et al.*[30]. Tissue homogenate of liver and pancreas (100 µL) is mixed with 900 µL of 50 mM phosphate buffer and 100 µL of 30 mM H_2O_2. Absorbance was measured at 240 nm against blank withoutH_2O_2. Units of catalase activity are expressed as the amount of enzyme that decomposes 1.0 µM of H_2O_2 per minute. The specific activity was expressed in terms of U/mg of protein.

Statistical Analysis

All statistical comparisons between groups were performed with One-way ANOVA followed by the Bonferroni method for independent observations using Graph Pad Prism 6. Differences were considered statistically significant at $P \leq 0.05$.

Results and Discussion

As a traditional herbal medicine, *Aloe vera* has been extensively studied[10] for various biological activities such as antioxidant, anti-inflammatory, antidiabetic, anticancer, etc. The antioxidant and anti-inflammatory activity of *A. vera* carbohydrates studied by different authors is reported individually. Whereas, our results suggest the possible correlation between the antioxidant and anti-inflammatory activity of *A. vera* carbohydrates data reports that *A. vera* polysaccharides can exhibit both activities *in vitro* and *in vivo* studies.

To identify the carbohydrate composition of the *Aloe* spp. polysaccharides several studies have been reported[31]. In this study, the monosaccharide composition of CF extract was analyzed using DIONEX CarboPac PA20 analytical column. It showed the presence of various monosaccharides like mannose, arabinose, galactose, glucose, rhamnose, xylose, fructose and disaccharide lactose with respect to standard retention time. Our results corroborate with earlier published literature[32]. However, some variation in monosaccharide composition has been observed which may be due to seasonal changes, geographic locations and age[33]. Qualitative analysis of CF of *Aloe vera* extract showed the presence of more amount of carbohydrates and traces of flavonoids, phenols, proteins[34]. After enrichment, the quantitative analysis of CF extract showed an increase in carbohydrate content with 180.9 mg/g when compared to *A. vera* extract (94.5 mg/g).

The CF extract was evaluated for antioxidant activity using free radical scavenging assays. Fig. 1 shows that at a maximum concentration of 2 mg/mL the CF extract display a significant (P <0.0001) free radical quenching potential of ABTS followed by a metal chelating activity, H_2O_2 and DPPH free radical scavenging activity in a dose-dependent manner. This potential antioxidant activity could be due to the donation of free electrons from its hydroxyl backbone to stabilize the oxygen atoms of free radicals which is

similar to the mechanism of scavenging property of carbohydrates[35].

It is known that the disturbance in equilibrium between reactive oxygen species (ROS) formation and antioxidant defense mechanism leads to oxidative stress associated damage of DNA, proteins and membrane lipids[36]. In heat-induced albumin denaturation assay, it was observed that the CF extract at a concentration of 2mg/mL showed maximum inhibition of 93.2% when compared to aspirin 86.4% (P <0.0001) (Fig. 2A). In membrane stabilization assay, the CF extract at a concentration of 2 mg/mL showed maximum protection by 72.7% against hypotonicity-induced hemolysis compared to diclofenac 63.7% (Fig. 2B). This result implies that CF extract may prevent protein denaturation and membrane lysis, tissue injury can be referred to as protein denaturation in cells and tissues[22]. Hence, inhibition of protein denaturation indicates the anti-inflammatory potential of CF extract.

The *in vitro* anti-inflammatory effect was studied using inflammatory markers such as NO, TNF-α and IL-6 levels in LPS stimulated RAW 264.7 cells. Nitric oxide production usually increases during inflammation through increased expression of inducible nitric oxide synthase (iNOS)[37]. It was observed that untreated macrophage RAW264.7 cells after stimulation with LPS induced immune response showed elevated levels of NO by 74.2% (Fig. 3A). After CF extract treatment at two different concentrations of 20 and 60 µg/mL, the LPS stimulated macrophage cells showed significantly decreased levels of NO production by 47.8% and 73.3% respectively (P <0.0001) compared to untreated LPS stimulated cells. It indicates that CF extract has better inhibition of NO production possibly due to controlled expression of iNOS which corroborates with the report on polysaccharides from the fruit of Sea buckthorn berry[38]. Lipopolysaccharide activation of extracellular regulated kinase (ERK1/2) via early growth response factor (Egr-1) promoter activity significantly increases TNF-α levels in LPS stimulatedmacrophages[39]. The untreated LPS stimulated macrophage showed markedly increased levels of TNF-α by 75.5%. The CF extract treatment significantly inhibited TNF-α production in murine RAW 264.7 macrophages by 46% and 73.2% respectively (P <0.0001) at the concentrations of 20 and 60 µg/mL when compared to untreated LPS stimulated macrophages (Fig. 3B). Our data suggest

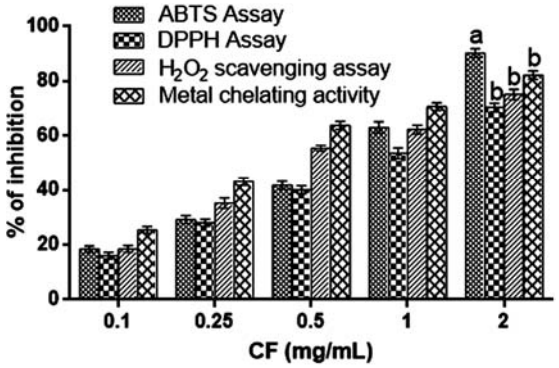

Fig. 1 — ABTS, DPPH, H_2O_2 free radical scavenging activities and metal chelating activity of CF extract. All values are expressed as Mean ± SD. Data were considered statistically significant (P <0.0001).

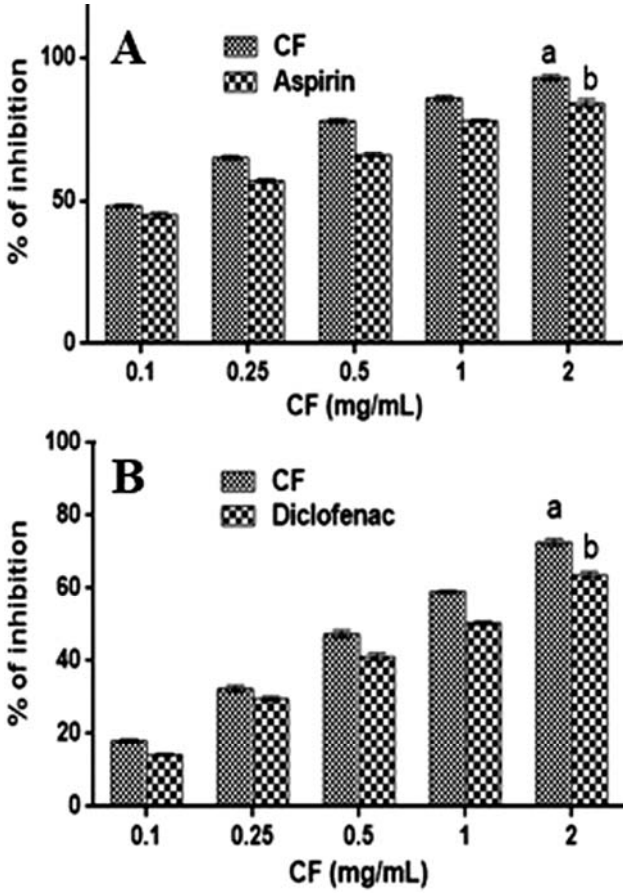

Fig. 2 — Assessment of *in vitro* anti-inflammatory activity of CF extract (A) inhibition of albumin denaturation; (B) Membrane stabilization assay. [All values are expressed as Mean ± SD. Data were considered statistically significant (P <0.01)]

that the CF extract may reduce the TNF-α level possibly by inhibition of (ERK1/2) activation and NF-κB signaling as similarly reported sulfated polysaccharide isolated from *Sargassum horneri*[40].

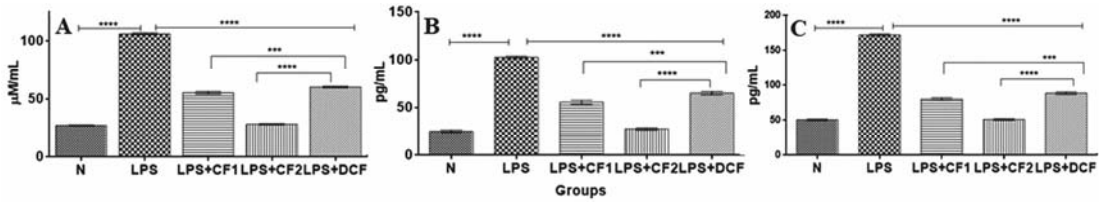

Fig. 3 — Effect of CF extract on LPS stimulated RAW264.7 macrophages on (A) inhibition of nitric oxide production; (B) TNF-α levels and (C) IL-6 levels. [Values are expressed as mean ± SD, n = 3, Significant differences of control cells compared to untreated LPS stimulated macrophages; **** P <0.0001.CF treatment on LPS stimulated macrophages at LPS+CF1 (20 µg/mL) and LPS+CF2 (60 µg/mL) compared to LPS stimulated macrophages (1 µg/mL);**** P <0.0001. Diclofenac (20 µM) treated LPS stimulated macrophages compared to untreated LPS stimulated macrophages;**** P <0.0001. CF treatment on LPS stimulated macrophages at LPS+CF1 (20 µg/mL) and LPS+CF2 (60 µg/mL) compared to diclofenac treated LPS stimulated macrophages; ***P <0.001; **** P <0.0001]

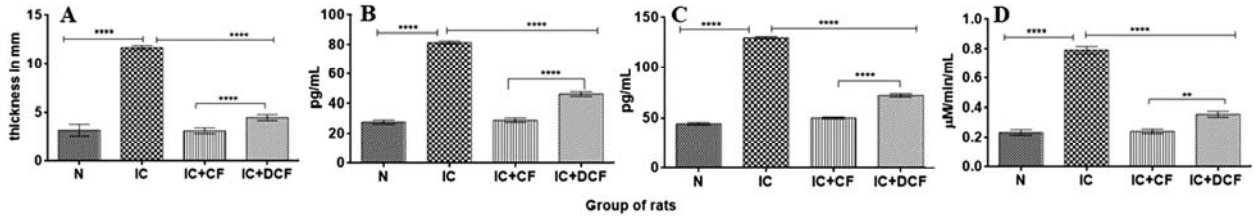

Fig. 4 — Effects of CF in Freund's adjuvant induced paw edema in rats (A) paw edema; (B) TNF-α levels; (C) IL-6 levels and (D) sPLA2 levels. [Values are expressed as mean ± SD, n = 6, Data were considered statistically significant for **** P <0.0001; ** P <0.01. Significant differences of normal rats compared to paw edema control rats. CF treatment (54 mg/kg) on paw edema rats compared to paw edema control rats. Diclofenac (10 mg/kg) treated paw edema rats compared to paw edema control rats. CF treatment on paw edema rats compared to diclofenac treated paw edema rats]

One of the functions of TNF-α is to induce the secretion of inflammatory cytokines such as IL-6, IL-1 during the inflammatory process[41]. TNF-α induction significantly increased IL-6 levels by 70.5% in untreated LPS stimulated macrophages (Fig. 3C). CF extract treatment at the concentrations of 20 & 60 µg/mL decreased the IL-6 levels significantly by 53.3% and 70.2% (P <0.0001) respectively compared to untreated macrophages. Our data suggest that a considerable decrease of these inflammatory markers signifies that CF extract from *Aloe vera* plays a major role in the immunomodulatory activity which is based on the stimuli generated in the immune response[13].

Complete Freund's adjuvant (CFA) induced rat paw edema model was used for the assessment of inflammatory markers/mediators such as TNF-α, IL-6 and s-PLA2 levels. The CFA-induced rat paw edema model is by cell-mediated autoimmunity due to structural imitation between cartilage proteoglycan and *Mycobacterium* induced rats[42]. It was noticed that a dose of 54 mg/kg bw of CF extract treatment showed no behavioural changes and no mortality rate in CFA-induced rat paw edema rats. The measurement of paw thickness of adjuvant induced rats (Gr. II) revealed an increase in diameter from day 7 up to day 14 by 73.9% (Fig. 4A). Increased paw swelling was found to be the result of edema of periarticular tissues with an increase in granulocytes and monocytes associated with changes in ankle diameter[43]. The CF extract-treated to adjuvant induced rats (Gr. III) significantly reduced the paw edema by 69.5 % when compared to arthritic control rats (Gr. II) (P <0.0001). This reduction of paw edema thickness may be probably due to inhibition of prostaglandin synthesis which is a principal inducing factor for acute inflammatory response[44]. The pathogenesis of inflammatory diseases is due to multiple inflammatory responses majorly by nuclear factor-κB which plays a significant role in synovial joint degradation and inflammation through the increase of TNF-α, IL-6 and sPLA2 levels. TNF-α, IL-6 act synergistically and activating the immune macrophage cells in the synovium and also involved in oxidative stress[5]. It was observed that in adjuvant induced paw edema rats the TNF-α, IL-6 and sPLA2 levels were observed high in plasma (70.1, 66.1 and 70.3%, respectively) (Gr. II) (Fig. 4 B-D). Upon treatment with CF extract to adjuvant induced paw edema rats the TNF-α, IL-6 and sPLA2 levels significantly reduced in the plasma by 64.1. II). This anti-inflammatory property of CF extract might be due to its various monosaccharides present in

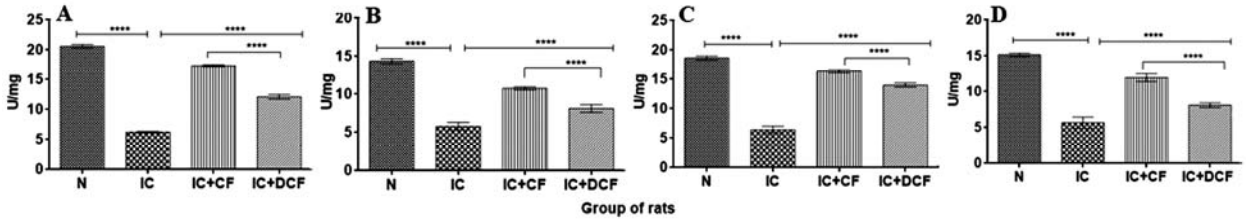

Fig. 5 — Effect of CF extract on antioxidant enzymes (A) liver SOD level; (B) liver catalase level; (C) pancreas SOD level and (D) pancreas catalase level. [Values are expressed as mean ± SD, n = 6, Data were considered statistically significant for ****P <0.0001. Significant differences of normal rats compared to paw edema rats. CF (54 mg/kg) treatment on paw edema rats compared to paw edema control rats. Diclofenac (10 mg/kg) treated paw edema rats compared to paw edema control rats. CF treatment on paw edema rats compared to diclofenac treated paw edema rats]

Aloe vera carbohydrates[45]. These results corroborate with the similar literaturereport[46] that polysaccharides have the potential to reduce the pathogenesis of inflammatory diseases through their anti-inflammatory efficacy by reduction of translocation of p65 subunit of NF-κB.

In the course of an increase in oxidative stress during inflammation, SOD and catalase act as a defense system against oxidative stress-based tissue injury, which interacts with superoxide toxicity and forms H_2O_2. Further catalase reacts with hydrogen peroxide to liberate water and oxygen with no free radical formation[47]. Reduced levels of these enzymes in paw edema rats showed that oxidative stress-based tissue damage plays a significant role in the pathogenesis of various inflammatory disorders[48]. The results obtained in our study showed that adjuvant induced paw edema control rats (Gr. II), enhanced oxidative stress. In the liver and pancreas, SOD and catalase levels were significantly decreased compared to normal rats (73.7 62.3 and 67.2 64.9%, respectively). After the administration of CF extract to adjuvant induced paw edema rats (Gr. III), significantly improved the liver and pancreas SOD, catalase levels by 64.1, 46.1% and 60.7, 52.9%, respectively (Fig. 5 A-D). This scavenging property of CF of *Aloe vera* decreases ROS/RNS levels and inflammatory mediators, such as TNF-α, IL-6, NO and $sPLA_2$ which might be primarily activated due to NF-kB and Active protein-1 during inflammatory response induced by lipopolysaccharide stimulation in RAW 264. 7 cells and Freund's adjuvant induced rat paw edema[49,50].

Conclusion

The results from our study indicate that the carbohydrate fraction (CF) of *Aloe vera* can scavenge free radicals and prevents cell membrane lysis. CF reduces the inflammatory mediators both in *in vitro* and *in vivo* models with an increase in antioxidant enzymes. The increase of SOD and catalase levels in adjuvant induced rats reduces inflammatory mediators possibly due to downregulation of the p65 subunit of NF-κB which exhibits low mitogenic response and inhibition of complement system in immune macrophage cells. Moreover, further studies are required to understand the mechanisms at cellular and molecular level and to identify the composition through purification and characterization studies of CF of *Aloe vera*. In conclusion, this study suggests that CF *Aloe vera* has promising potential in the management of oxidative stress and inflammatory disorder.

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Conflicts of interest

Authors declare no conflict of interests.

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