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Effect of Enteral Administration of α – Linolenic Acid and Linoleic Acid against Methotrexate Induced Intestinal Toxicity in Albino Rats

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Abstract: The present study was confronted to exemplify the effect of α- Linolenic acid (ALA) (18:3, ω-3) and Linoleic acid (LA) (18:2, ω-6) on experimental intestinal toxicity induced by methotrexate (MTX). The groups of albino rats received, Group I: normal saline (2 ml/kg, i.p. sham control), Group-II : MTX (2.5 mg/kg, i.p. toxic control); Group-III: ALA (2 ml/kg, i.p.); Group-IV: LA (18:2, ω-6) (2 ml/kg, i.p.), Group-V : ALA (2 ml/kg, i.p.) and Group-VI: LA (2 ml/kg, i.p.) with MTX (2.5 mg/kg, i.p.). Animals were sacrificed after 7 days treatment schedule and appraised for intestinal pH, total acidity, free acidity and colonic mucosal disease index (CMDI). Intestinal tissues were further evaluated for oxidative stress parameters (TBARS, SOD, protein carbonyl and catalase), and morphological modulation using scanning electron microscopy. The intestinal tissues were further graded for the enzymatic activities of COX-1, COX-2 and 15-LOX. Both ALA and LA demonstrated momentous protection against MTX induced intestinal toxicity, which could be attributed to their prooxidant nature.

Key words: α- Linolenic acid, COX-1, COX-2**,** Intestinal toxicity, Linoleic acid, 15-LOX, Methotrexate, Oxidative stress

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Abbreviations:

AA: Arachidonic Acid

ALA: α- Linolenic acid

CMDI: Colonic mucosal disease index

DAI: Disease activity index

DHA: Docosahexanoic Acid

EPA: Eicosapentanoic Acid

EFA: Essential Fatty Acid

LA: Linoleic acid

MTX: Methotrexate

PUFA: Polyunsaturated fatty acid

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Introduction:

Methotrexate (MTX) is a robust anticancer drug, anatomically similar to folic acid and impedes the dihydrofolate reductase enzyme which disciples the dihydrofolic acid to tetrahydrofolic acid. The competence of MTX is limited by relentless side effects and toxic effects including intestinal injury and enterocolitis [1]. MTX restrained causes the devastation of intestinal mucosa and perturbs the barrier against intravascular bacteria leading to relentless inflammation followed by derogation and ulceration of intestine and colon [2]. MTX induced inflammatory reactions motivates impairment of antioxidant defence mechanism and accomplish the tissue more receptive to oxidative damage due to fructification of reactive oxygen species (ROS) [3]. One of the most prevalent pharmacological approach to countervail the MTX induced intestinal toxicity is to combat inflammatory pathway by consolidating down the biosynthesis of pro-inflammatory eicosanoids particularly derived from arachidonic acid (AA) (20:4, ω-3).

Essential Fatty acids (EFA) are fatty acids that human and other animals cannot synthesize and obtained from diet. The α-Linolenic acid (ALA) (18:3, ω-3) and Linoleic acid (LA) (18:2, ω-6) are the two such fatty acids, paramount for humans, retaining array of role in physiological system [4]. LA (18:2, ω-6) is indoctrinated into gamma-linolenic acid (GLA) (18:3, ω-6) in body, which is further constituted to AA (20:4, ω-3). AA (20:4, ω-3) sits on the top of the inflammatory cascade with more than 20 different signalling pathways and governs a wide array of body functions including inflammatory cascade [5.] In divergence to the fact that GLA (18:3, ω-6) is one of the intermediate molecule for synthesis of AA (20:4, ω-3), the previous disquisitions proponed that GLA (18:3, ω-6) plays an important role in allocating inflammation [6]. Recently, it was ascertained that GLA (18:3, ω-6) preclude the switching of inflammatory cytokines by reconciling nuclear factor kappa β (NF-kβ). GLA (18:3, ω-6) also bring to bear its anti-inflammatory effects by promoting the pervasive peroxisome proliferator activated receptor (PPAR) system [7]. GLA (18:3, ω-6) has also flaunted great affirmation in overseeing symptoms of rheumatoid arthritis [8]. Two other essential fatty acids integrate a cascade that runs alongside and emulates with the AA (20:4, ω-3) cascade, eicosapentanoic acid (EPA) (20:5 ω-3) and decosahexanoic acid (DHA) (22:6 ω-3). The EPA (20:5 ω-3) provides the most conspicuous competing cascade. EPA (20:5 ω-3) and DHA (22:6 ω-3) are ingested from fish oils or derived from dietary ALA (18:3, ω-3) by a series of desaturation and elongation reactions [9]. Foregoing studies take account of that EPA (20:5 ω-3) cascade softens the inflammatory effects of AA (20:4, ω-6) cascade, hence, materializing

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as an anti-inflammatory agent [10]. As particularized above, GLA (18:3, ω-6) /AA (20:4, ω-6) and EPA (20:5 ω-3)/DHA (22:6 ω-3) are the commodities of LA (18:2, ω-6) and ALA (18:3, ω-3) metabolism respectively and exhibit wavering pharmacological actions [6]. Therefore a scientific predicament exists over against delineating the anti-inflammatory potential/mechanism of ω-3 and/or ω-6 fatty acids. It would be credible that recently our laboratory has reported the significant *in-vitro* and *in-vivo* anti-inflammatory activity of LA (18:2, ω-6) and ALA (18:3, ω-3) [6]. Moreover, the anti-inflammatory, anti-arthritic along with anti-ulcer activity of *Linum usitatissimum* fixed oil has been reported and the aforesaid was urged to be interceded through dual inhibition of AA (20:4, ω-3) metabolism by ALA (18:3, ω-3) (a major constituent in oil) [11]. Through the similar series of work has also manifested the presence of momentous amount of LA (18:2, ω-6) (precursor for AA (20:4, ω-6) synthesis) present in the oil as well. In view of the reports from our laboratory as well as from the others, one can derive that there is paucity of plentiful scientific evidences towards this aspect of PUFA research. Considering the above and in gist of particularizing the physiological role of ω-3 and ω-6 EFA, the present work has been undertaken to investigate the effect of LA (18:2, ω-6) and ALA (18:3, ω-3) against MTX induced intestinal toxicity in albino rats.

Materials and Methods:

Drug and chemicals

ALA (18:3, ω-3), LA (18:2, ω-6) (*Rolex Chemical Industries*, Mumbai, India) and MTX (Folitrax-15, *Ipca Pharmaceuticals Ltd*. Mumbai, India) were purchased from the local market. The ELISA kits for COX-1, COX-2 (catalogue no. 760111) and 15 LOX (catalogue no. 760700) were procured from *Cayman Chemicals* Ltd USA. All other chemicals were procured from *Hi-media* Mumbai, India and were of analytical grade.

In vitro antioxidant assay:

DPPH radical scavenging activity: Methanol solution accommodating ALA (18:3, ω-3) and LA (18:2, ω-6) (20 – 120 µg/ml for each separately) mixed with DPPH solution (100 µM in methanol) and incubated at 37 ˚C for 30 min. After incubation the absorbance of the reaction mixture was read at 517 nm using UV–visible spectrophotometer (*Labtronics* – LT – 2910 Double Beam) [12]. The experiment was performed in triplicate.

H_2O_2 scavenging activity: Spectrophotometric method was used to resolve the competency of ALA (18:3, ω-3) and LA (18:2, ω-6) to quench H_2O_2. Divergent concentration of ALA (18:3, ω-3) and LA (18:2, ω-6) (20 – 120 µg/ml for each separately) were dissolved in 0.1

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M, pH 7.4 phosphate buffer and mixed with 40 mM solution of H_2O_2. Absorption of H_2O_2 at 230 nm was determined 10 min later in UV–visible spectrophotometer (*Labtronics* – LT – 2910 Double Beam). A separate blank sample was used for background subtraction. The experiment was performed in triplicate [12].

Animals:

Albino wistar rats (120-150 gm) of both sexes were retrieved from the central animal house facility. The albino rats were kept in polypropylene cage under standard condition of temperature (22 ± 5˚C) with 12 h light/dark cycle with free access to a commercial pellet diet and water. The experimental protocol was endorsed by Institutional Animal Ethics Committee (IAEC) (approval no. UIP/IAEC/2014/Feb/08). Animals were randomized and divided into 6 groups of 6 animals each. Group I (sham control, 0.9% normal saline i.p.); Group II (toxic control, MTX 2.5 mg/kg i.p.); Group III (ALA 2 ml/kg i.p.), Group IV (LA 2 ml/kg i.p.); Group V (MTX+ALA 2.5 mg/kg + 2 ml/kg i.p.); Group VI (MTX+LA 2.5 mg/kg + 2 ml/kg i.p.)[1,6]. Toxicity was induced by single i.p. injection of MTX followed by ALA and LA supplementation therapy for seven days at the dose prescribed above. Animals were sacrificed on 8th day and subjected to estimation.

Evaluations:

Estimations of pH, free acidity and total acidity: After the respective treatment animals were euthanized with cervical dislocation and the intestinal tissue was collected. The content of the intestinal tissue was collected and evaluated for intestinal pH using pen type pH meter (*Hanna Instrument* HI 98107). Free acidity and total acidity were appraised by endorsing the procedure described previously. Total acidity and free acidity was expressed as mEq/l [13, 14].

Assessment of CMDI: The colon tissue of approximately 10 cm to anus was taken, opened longitudinally and washed in normal saline buffer and fixed on wax block. The scoring was done and evaluated by using the formula of CMDI represented as follow. 0 = normal mucosa, 1= mild hyperemia, no erosion or ulcers on the mucosa surface, 2 = moderate hyperemia, erosion or ulcers appears on the mucosa surface, 3 = sever hyperemia, necrosis and ulcers on the mucosa surface with the ulcerative area less than 40%, 4 = sever hyperemia, necrosis and ulcers on the mucosa surface with the ulcerative area more than 40% [15].

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Biochemical estimation: The distal part of intestinal tissues (10% w/v) were homogenised in 0.15M KCL at 10,000 rpm (4°C). The supernatants were scrutinized for the biochemical parameters include TBAR's [16], SOD [17], protein carbonyl [18] and catalase [19] using the methods established at our laboratory [20, 21].

COX-1, COX-2 and 15-LOX: The supernatants as collected above were further appraised for the enzymatic activities of COX-1, COX-2 and 15 LOX using commercial ELISA kits from *Cayman Chemicals* Ltd USA, as per the method described by the manufacturer using microplate reader (*Alere Microplate Reader* AM, 2100).

Morphological evaluation: The intestinal tissues from all the groups were evaluated for their morphological changes using scanning electron microscopy. Samples were fixed in 2.5% glutaraldehyde for 6 h at 4˚C and washed with 0.1 M phosphate buffer, for 3 changes each of 15 min at 4˚C. 1% osmium tetraoxide was used as a post fixation for 2 h at 4˚C and samples were washed in 0.1 M phosphate buffer for 3 changes each of 15 min at 4˚C to remove the uncreative fixative. Specimens were dehydrated by using increasing concentration of acetone viz. 30%, 50%, 70%, 90%, 95%, 100% (dry acetone) to remove water at 4˚C for 30 min period. After that, samples were air dried (critical point i.e. 31.5 at 1100 psi). The specimens were mounted on to the aluminium stub with adhesive tape and the specimens were observed in scanning electron microscope (JEOL-JSM-6490LV).

Statistical analysis: All data were presented as mean ± SD and analyzed by one way ANOVA followed by Bonferroni test for the possible significance identification between the various groups. $*P<0.05$, $**P<0.01$, $***P<0.001$ were considered statistically significant. Statistical analysis was carried out using Graph pad prism (3.2), San Diego, California.

Results: The intraperitonial administration of ALA (18:3, ω-3) and LA (18:2, ω-6) incomparably inhibited the intestinal toxicity in the experimental animals illustrated through, conspicuous reduction in the free acidity (26.15%) total acidity (22.35%) and CMDI (83.25%) in analogy to control (Table 1). The treatment with the LA (18:2, ω-6) also afforded a momentous protection in contrast to MTX induced toxicity, however the same was perceived to be inconsiderable in comparison to ALA (18:3, ω-3).

MTX made evident a compelling upsurge in fructification of MDA (9.70±0.37 nM of MDA/ mg of protein).The treatment group with LA (18:2, ω-6) and ALA (18:3, ω-3) bestowed a momentous protection from the same, just about uniformly (Table 2). When scrutinized for

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the protein oxidation, the MTX treatment evidenced compelling increase in protein carbonyl levels in toxic groups (116.81±0.68 nanomoles/ml) in resemblance to normal control (50.60 ±4.17 nanomoles/ml). Concomitant administration of LA (18:2, ω-6) and ALA (18:3, ω-3) re-established the protein carbonyl to a significant level. Similarly, outstanding increase in SOD was contemplated in the toxic control (43.99±7.86 SOD/mg of protein) in counterpart to sham control (29.63±2.51 SOD/mg of protein) (Table 2). On the discordant compelling subsidence in the enzymatic activity of catalase was perceived in toxic control (8.27±1.45 nM of H_2O_2/min/mg of protein), it is noteworthy that treatment with ALA (18:3, ω-3) and LA (18:2, ω-6) helped to restore the enzymatic activities of SOD and catalase synchronously.

The intestinal tissue evidenced compelling rise in the enzymatic activity of COX-1 and COX-2 after the concomitant administration of ALA (18:3, ω-3) and LA (18:2, ω-6) with MTX, discordantly 15-LOX activity was contemplated to be decreased in ALA (18:3, ω-3) and marked up in LA (18:2, ω-6) treated group when administered concomitantly with MTX (Table 3).

When monitored morphologically sententious abnormalities in the mucosa of treated rats were detected including hyper-proliferation, progressive distortion of the crypts, mucosal surface irregularities suggesting derogation and formation of focal protuberances was monitored in the MTX treated groups. The consequent administration of ALA (18:3, ω-3) and LA (18:2, ω-6) manifested a pronounced assurance against the same in a dose dependent manner (Figure 1).

The results from the DPPH and H_2O_2 scavenging assay depicts, no antioxidant property of ALA and LA. Rather the results reflect significant prooxidant nature of both the test compounds (Figure 2).

Discussion: MTX is an anti-cancer drug with anti-metabolite action and used as an anti-cancer and anti-rheumatic agent. The use of MTX is concorded with sizable number of toxicities, not to mention intestinal toxicity, cardiotoxicity, hepatotoxicity, nephrotoxicity and few more, circumscribing its expediency in the diversified malady [22]. In the present work we validated a remarkable assurance by ALA (18:3, ω-3) and LA (18:2, ω-6) against MTX induced intestinal toxicity.

Treatment with ALA (18:3, ω-3) and LA (18:2, ω-6) decidedly impeded the intestinal toxicity by regularizing the pH, decreasing the free acidity and total acidity in contrast to

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toxic control. The concomitant administration of ALA (18:3, ω-3) and LA (18:2, ω-6) with MTX also slackened the CMDI to a convincing level. The above perceived effects of ALA (18:3, ω-3) and LA (18:2, ω-6) are in concordance with the antecedent reports and the same could be imputed to the anti-histaminergic (anti-secretary) and anti-cholinergic (anti-secretary and vasodilator) effects of PUFA. The anti-secretary effects of ALA (18:3, ω-3) and LA (18:2, ω-6) could be imputed to the muscarinic 3 and histaminergic 2 antagonistic actions as proclaimed previously [23]. The ALA (18:3, ω-3) displayed surpassing assurance against the intestinal toxicity in collation to LA (18:2, ω-6).

The heightened production of the MDA and protein carbonyl is the unambiguous markers for oxidative damage to the lipids and proteins respectively [24]. Although there is a no secluded universal marker for protein oxidation, however protein carbonyl appraisal is extensively accustomed as a marker for protein oxidation [25]. We scrutinized convincing upsurge in the protein carbonyl content in the toxic group which was re-established after the concomitant administration of ALA (18:3, ω-3) and LA (18:2, ω-6). It is worth to mention that ALA (18:3, ω-3) substantiated inappreciably appropriate conservation towards protein oxidation in analogy to LA (18:2, ω-6). The MTX treatment in the toxic control group designated compelling increase in the procreation of MDA products and thereby pointing the concurrence of lipid per-oxidation in the MTX toxicity, which is in corroboration with the previous proceeding [26]. Concomitant administration of ALA (18:3, ω-3) and LA (18:2, ω-6) depreciated the levels of MDA products to a significant level and by that curtailed oxidative stress.

The SOD and catalase together complement an extensive defence team against the ROS, the SOD abrogate the superoxide free radical to form hydrogen peroxide which after while is neutralized by a heam protein, catalase [20]. Catalase recede the hydrogen peroxide to engender water and molecular oxygen. Both the enzymes work in tendon to protect the tissue from highly reactive free radicals [27]. In the present experiment we observed a momentous increase in the SOD enzyme, further affirming the concurrence of ROS and accompanying administration of ALA (18:3, ω-3) and LA (18:2, ω-6) significantly restored the enzymatic activity of SOD. The synchronic increase in the enzymatic activity of catalase is expected with increase in SOD and is reputed extensively as well [21]. However this was not replicated in our experiment and momentous decrease in tissue catalase was evidenced after MTX administered. Notwithstanding, the therapeutic regimen of ALA (18:3, ω-3) and LA (18:2, ω-6) fizzled to rehabilitate the slackened levels of catalase.

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The MTX associated chemotherapy has been proclaimed to instituted the mucositis directly by provoking DNA strand break through the generation of ROS, ROS may outrage other cell and tissues and prompt the secondary mediators including NF-Κβ and pro-inflammatory cytokines [28, 29]. The rousing of transcription factor (NF-Κβ) in counter to ROS, further results in the gene up regulation for TNF-α, interleukins (IL-1β, IL-6) leading to injury and apoptosis not beyond the submucosal and basal epithelium [30]. The inflamed intestine manifest the subsistence of oxidative stress leading to oxidation of lipids; proteins and DNA damage [3]. Therefore, the MTX lured toxicity is conspicuous by the increased enzymatic activity of COX-1, COX-2 and 15-LOX as experienced in the current experiment. The ALA (18:3, ω-3) and LA (18:2, ω-6) were preceded to farther increase the COX-1and COX-2 activity, whereas the 15-LOX was re-established to routine incomparably. This could be interpreted with the certitude that ALA (18:3, ω-3) and LA (18:2, ω-6) are metabolized to AA (20:4, ω-6) and EPA (20:5, ω-3) consequently. Both ALA (18:3, ω-3) and LA (18:2, ω-6) are the substrate for COX-1, COX-2 and 15-LOX [6]. Moreover, ALA (18:3, ω-3) and LA (18:2, ω-6) have forthright inhibitory effect on COX-1 and COX-2 only as reputed by antecedent studies [6]. Thus, we derive that due to increased substrate availability for COX-1 and COX-2 followed by direct inhibitory effect of ALA (18:3, ω-3) and LA (18:2, ω-6); the rebuttal mechanism could have inseminated the increase enzymatic activity of COX-1 and COX-2 as ascertained in our experiment. It would be pertinent to mention that previous report suggest the direct inhibitory activity of ALA (18:3, ω-3) and LA (18:2, ω-6) against cyclooxygenases, without too much affecting the lipoxygenase [4]. Due to paucity of forthright inhibitory effect of ALA (18:3, ω-3) and/or LA (18:2, ω-6) on LOX, we observed the restoration in the enzymatic level of 15-LOX.

When contemplated microscopically considerable hyperproliferation and mucosal degeneration was preceded in MTX treated experimental animal, which is consonance with the previous studies. Both ALA (18:3, ω-3) and LA (18:2, ω-6) manifested significant microscopic protection contrary to the MTX induced intestinal toxicity in experimental animals.

The *in-vitro* anti-oxidant activity of the ALA (18:3, ω-3) and LA (18:2, ω-6) was appraised contrary to DPPH and H_2O_2 assay. DPPH is a steady free radical, whereas H_2O_2 is highly reactive and consequently short lived. The H_2O_2 in every molecule in collation to DPPH have proficiency to catastrophe almost every molecule in a living cell [31]. The ALA (18:3, ω-3) and LA (18:2, ω-6) demonstrated robust pro-oxidant activity against DPPH and H_2O_2 assay

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suggesting their ability to interact with the wide range of free radicals. The pro-oxidant activity of the ALA (18:3, ω-3) and LA (18:2, ω-6) as perceived in present experiment can be imputed to the high degree of unsaturation present in the ALA (18:3, ω-3) and LA (18:2, ω-6) [32].

As discussed above the metabolic products of LA (18:2, ω-6) are pro-inflammatory, whereas that of ALA (18:3, ω-3) are anti-inflammatory. However both contributed a sententious physiological, biochemical and morphological protection against the MTX induced toxicity. The same could be elucidated, that the exogenic supplementation of ALA (18:3, ω-3) and LA (18:2, ω-6) (pro-oxidant unsaturated FA), would have accomplished themselves receptive for incursion by ROS, engendered through MTX toxicity. Henceforth, we postulate/hypothesize that both ALA (18:3, ω-3) and LA (18:2, ω-6) have demonstrated significant protection due to their competence for being a prooxidant, which could be attributed to their polyunsaturated nature, making them susceptible for ROS attack. It would be worth to remark that ALA (18:3, ω-3) demonstrated somewhat sharpened protection in exemplification to LA (18:2, ω-6) and the aforesaid could be imputed to the generation of anti-inflammatory mediators in comparison pro-inflammatory mediators from LA (18:2, ω-6). Authors would also like to comment that the preservation demonstrated by LA (18:2, ω-6) may be lost in long term therapeutic regimens due to generation of pro-inflammatory metabolites of AA (20:4, ω-6).

It can be conclude that ALA (18:3, ω-3) and LA (18:2, ω-6) could be used as an adjuvant with MTX chemotherapy and/or clinical management of arthritis. These therapeutic effects as derived through current experimental evidences can be attributed to their action on oxidant-antioxidant systems and inflammation process. However the clinical significance of ALA (18:3, ω-3) and LA (18:2, ω-6) in various clinical pathologies has been a matter of debate and different scientist across the world has opined differently on this issue. Therefore further experimental and clinical studies are required to ascertain these findings.

Conflict of interest:

No conflicts of interest

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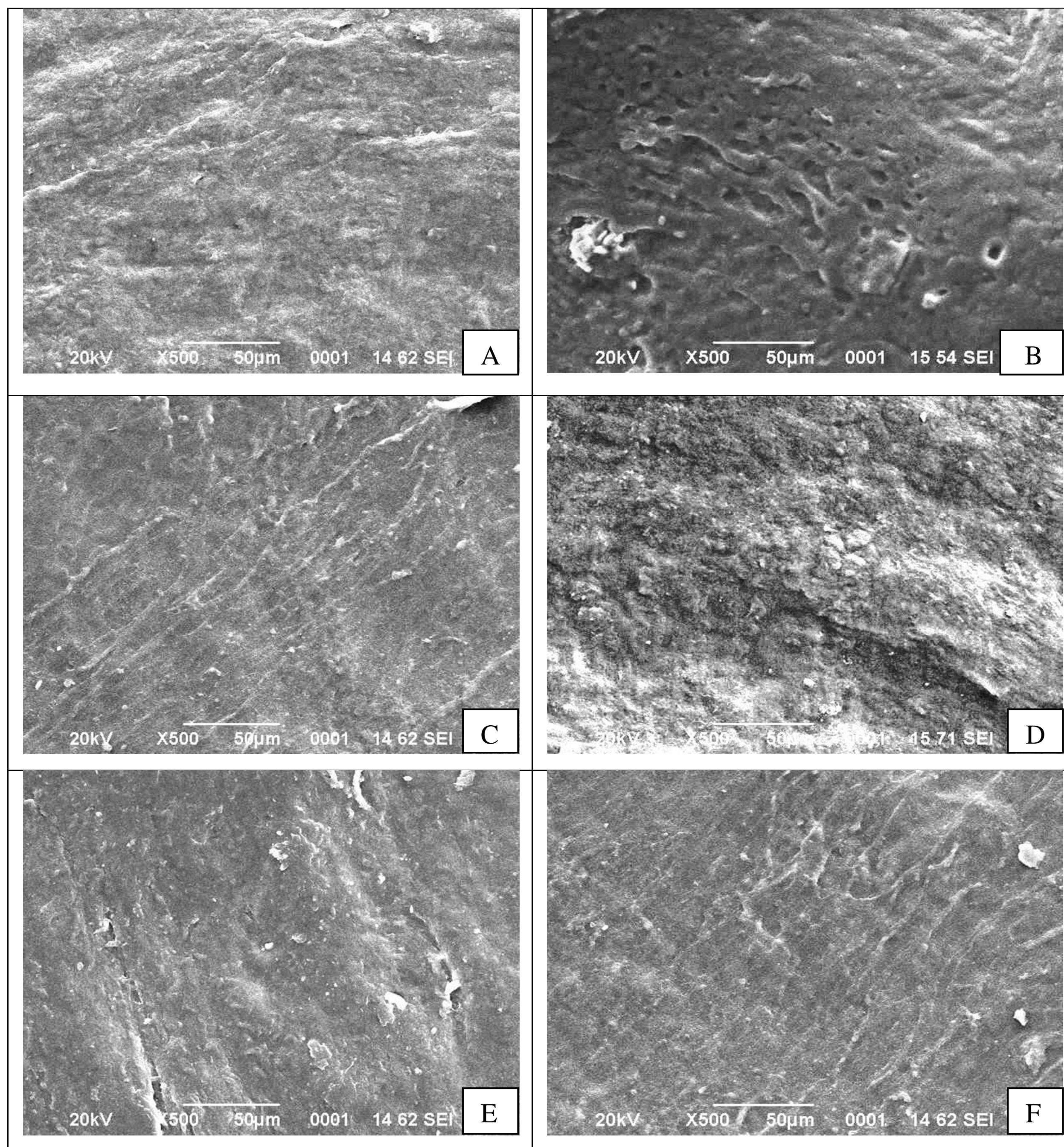

Figure 1:- (A) Sham Control (Normal Saline 2ml/kg i.p.); (B) Toxic Control (MTX 2.5 mg/kg i.p.); (C) ALA (2 ml/kg i.p.); (D) LA (2ml/kg i.p.); (E) MTX+ALA (2.5 mg/kg + 2 ml/kg i.p.); (F) MTX+LA (2.5 mg/kg + 2 ml/kg i.p.)

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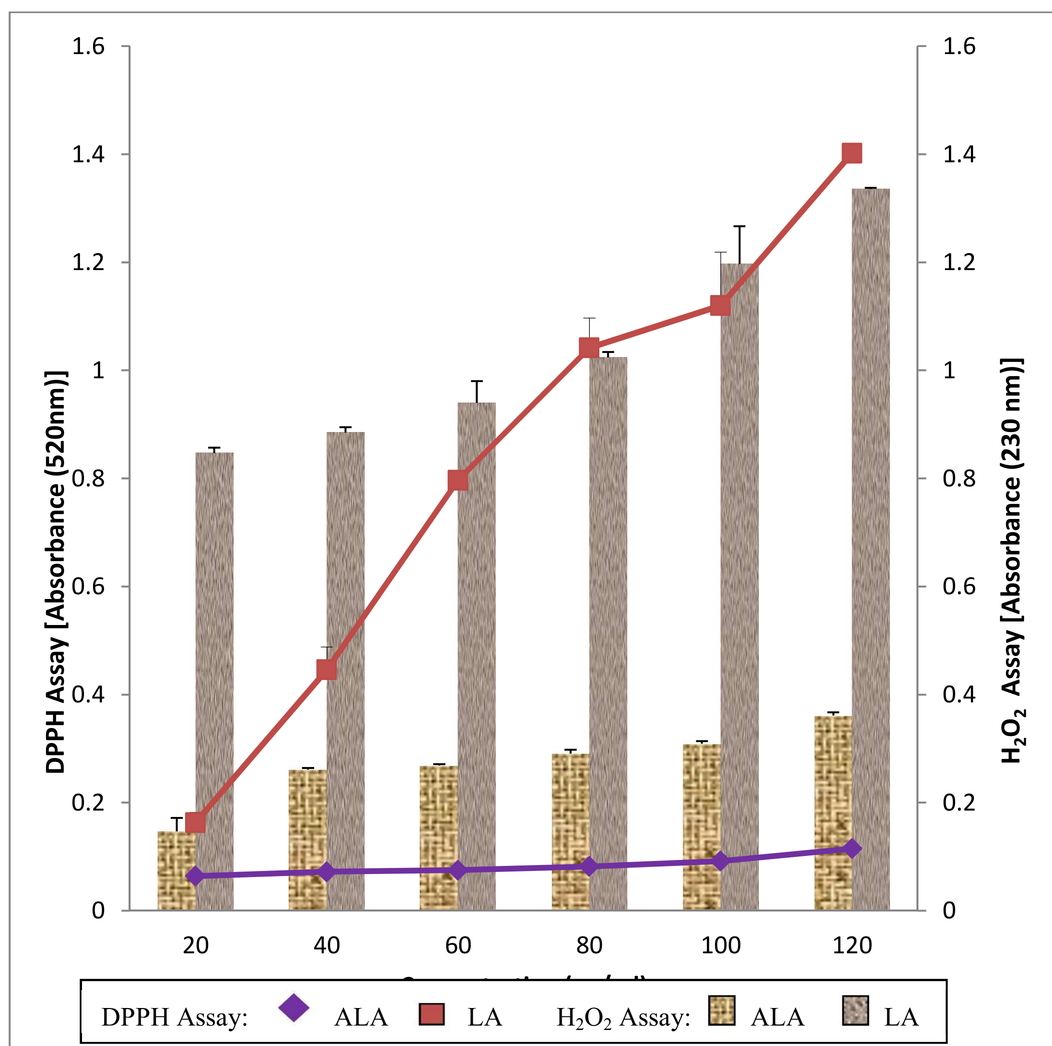
Figure 2: Invitro antioxidant activity of ALA and LA using DPPH and hydrogen peroxide assay



Data represented as mean ± SD (n=3)

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Table 1. Effect of ALA and LA therapy on intestinal pH, free acidity, total acidity and CMDI.

Groups	Treatment (i.p.)	Intestinal pH	Free acidity (mEq/l)	Total acidity (mEq/l)	CMDI
Group I	Sham control (Normal saline, 2 ml/kg)	5.28±0.23***	10.59±0.69***	13.58±0.61***	0.00±0.00***
Group II	MTX (Toxic control) (2.5 mg/kg)	4.55±0.27	17.32±2.23	20.71±0.81	4.00±0.00
Group III	ALA (2 ml/kg)	5.20±0.17***	10.59±0.49*** (38.85%)	14.29±0.46*** (30.99%)	0.00±0.00*** (100%)
Group IV	LA (2 ml/kg)	5.47±0.14***	12.20±1.08*** (29.56%)	15.01±0.35*** (27.52%)	0.50±0.84*** (87.5%)
Group V	MTX + ALA (2.5 mg/kg + 2 ml/kg)	5.25±0.19***	12.79±0.78*** (26.15%)	16.08±0.28*** (22.35%)	0.67±0.81*** (83.25%)
Group VI	MTX + LA (2.5 mg/kg + 2 ml/kg)	5.37±0.15***	13.96±0.78*** (19.39%)	16.05±0.77*** (22.50%)	1.00±0.89*** (75%)

Each group contains six animals; Data is represented as Mean ± SD,
Statistical significance compared to toxic control using one-way ANOVA followed by Bonferroni test.
$P<0.05$, $**P<0.01$, $***P<0.001$ were considered statistically significant.
Values in parenthesis represent percentage inhibition.

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Table 2. Effect of ALA and LA therapy on TBAR, Protein carbonyl, SOD and Catalase on intestinal tissue.

Groups	Treatment (i.p.)	TBAR (nM of MDA/ mg of protein)	Protein Carbonyl (Nano moles/ml)	SOD (SOD/mg of protein)	Catalase (nM of H_2O_2/min/mg of protein)
Group I	Sham control (Normal saline, 2 ml /kg)	8.18±0.13***	50.60±4.17***	29.63±2.51***	12.60±2.15**
Group II	MTX (Toxic control) (2.5 mg/kg)	9.70±0.37	116.81±0.68	43.95±7.86	8.27±1.45
Group III	ALA (2 ml/kg)	8.32±0.17***	50.45±1.60***	29.68±1.85***	10.18±2.62
Group IV	LA (2 ml/kg)	8.33±0.39***	37.57±5.34***	30.68±1.85***	8.22±0.92
Group V	MTX + ALA (2.5 mg/kg + 2 ml/kg)	8.72±0.17***	50.84±4.17***	38.66±1.50***	8.25±0.73
Group VI	MTX + LA (2.5 mg/kg + 2 ml/kg)	8.71±0.27***	49.85±0.35***	37.31±2.21***	8.38±0.86

Each group contains six animals; Data is represented as Mean ± SD,
Statistical significance compared to toxic control using one-way ANOVA followed by Bonferroni test.
$P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ were considered statistically significant.

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Table 3: Effect of ALA and LA therapy on Cyclooxygenase and Lipoxygenase activity in intestinal tissue

Groups	Treatment (i.p.)	COX-1 (µmol/ml/min)	COX-2 (µmol/ml/min)	15-LOX (µmol/ml/min)
Group I	Sham control (Normal saline, 2 ml /kg)	29.40±2.00	24.22±1.99**	13.54±2.26***
Group II	MTX (Toxic control) (2.5 mg/kg)	34.32±3.35	28.99±0.74	37.08±7.88
Group III	ALA (2 ml/kg)	36.86±8.68	18.42±0.07***	14.69±1.13***
Group IV	LA (2 ml/kg)	47.79±5.97*	24.05±1.13***	17.47±3.90**
Group V	MTX + ALA (2.5 mg/kg + 2 ml/kg)	52.55±2.28**	25.37±0.00*	18.64±3.73**
Group VI	MTX + LA (2.5 mg/kg + 2 ml/kg)	56.32±3.00**	23.72±0.28***	15.02±0.00***

Each group contains six animals; Data is represented as Mean ± SD,
Statistical significance compared to toxic control using one-way ANOVA followed by Bonferroni test.
$^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ were considered statistically significant.

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