



Modification of allergenicity and immunogenicity of formate dehydrogenase by conjugation with linear mono methoxy poly ethylene glycol: Improvement in detoxification of formate in methanol poisoning

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Abstract

Background: Single bolus intravenous infusion of native formate dehydrogenase (FD), isolated from *Candida boidinii* was found to eliminate formate, a highly toxic metabolite in methanol poisoning. In order to prevent immunological reactions which might be produced by multiple dosing of formate dehydrogenase and to prolong the serum half life of the enzyme, the *N*-hydroxysuccinimidyl ester of methoxy polyethylene glycol propionic acid (mPEG-SPA 5000) was conjugated to native formate dehydrogenase.

Method: PEGylation reactions were run at 20 °C for 30 min in a reaction buffer (0.2 mol/l sodium phosphate buffer, pH 8.3). The PEGylated molecules were purified from unreacted PEG with Amicon Ultra-4 (10 K) and by Sephacryl S-300 HR gel-filtration chromatography. Unreacted formate dehydrogenase molecules were removed by DEAE Sepharose FF anion-exchange chromatography. PEG–FD enzyme molecules obtained from reacting ratio of FD/PEG of 1/40 had an enzyme activity of 68% of unmodified enzyme. Immunogenicity of PEGylated and native enzyme was evaluated by ELISA. Allergenicity was evaluated by active systemic anaphylaxis and passive cutaneous anaphylaxis tests. In vivo efficacy of PEG–FD or native FD was comparatively evaluated by single intravenous administration of PEG–FD or native FD in folate deficient methanol intoxicated albino rats along with Carbicarb buffer infusion. Methanol and formate were estimated at specific time points respectively with HPLC and fluorescence spectrophotometer.

Result: PEG–FD had comparatively longer half life and lower immunogenicity than native FD. PEG–FD had better in vivo efficacy than native FD in eliminating the formate.

Conclusion: Conjugation of mPEG-SPA 5000 with native FD reduces its immunogenicity and increases its efficacy in detoxification of formate in methanol poisoning.

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Keywords: PEGylation; Formate dehydrogenase; Formate; Methanol; Immunogenicity

1. Introduction

Methanol is well absorbed from the gastrointestinal tract mucosa as well as through the skin and lungs. Apart from oral ingestion, both inhalation and transdermal exposure can result in toxicity. It should be noted that the highest morbidity and

mortality have been associated with deliberate or accidental oral ingestion of methanol-containing mixtures [1]. It is one of the major adulterants of illicit liquor in India causing blindness and death [2]. In mammalian species, methanol is metabolized to formaldehyde in the liver and by subsequent oxidative steps, formic acid and carbon dioxide are formed [3,4]. The metabolism of formate is mediated through folate dependent pathway [5]. Humans (and non-human primates) are uniquely sensitive to methanol poisoning because of their low liver folate content [6]. Specifically, formic acid is the toxic metabolite responsible for the metabolic acidosis observed in methanol poisoning in humans and in folate-depleted rodents [4]. Formic acid is believed to be

Abbreviations: FD, formate dehydrogenase; PEG FD, pegylated formate dehydrogenase; i.p., intra peritoneum; FDD, folate deficient diet; MeOH, methanol; Carb, Carbicarb; PBS, phosphate buffered saline; mPEG, mono methoxy polyethylene glycol.

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the toxic metabolite responsible for the ocular toxicity in methanol-poisoned humans [7].

Formate dehydrogenase (EC 1.2.1.2) acts directly by converting formate into CO₂ in the presence of NAD. Efficacy of single bolus intravenous administration of native formate dehydrogenase, isolated from the *Candida boidinii*, in detoxification of formate, in methanol poisoning was reported in our earlier study [8].

Chemical modification of therapeutic proteins with mono methoxy poly ethylene glycol (mPEG) to reduce the immunogenicity and to increase the circulating half life has been extensively studied [9–11]. Asparaginase modified with mPEG, is accepted by FDA, in patients with immunological reaction towards the free enzyme therapy [12]. In this study, formate dehydrogenase was modified with linear *N*-hydroxysuccinimide ester of mono methoxy polyethylene glycol propionic acid (mPEG-SPA 5000). The associated changes such as immunogenicity, allergenicity, plasma half life were evaluated and compared with that of native enzyme.

Rodents develop formate accumulation and acidosis similar to human beings in methanol poisoning, only when they are made folate deficient [13]. In vivo efficacy of PEG–FD or native FD was comparatively evaluated by single bolus intravenous administration of PEG–FD or native FD in folate deficient methanol intoxicated albino rats, along with Carbicarb buffer infusion. Carbicarb was used to treat the metabolic acidosis. Carbicarb is an alkalinizing agent, combination of 0.33 mol/l NaCl and 0.33 mol/l sodium bicarbonate [14–17]. Carbicarb besides correcting the metabolic acidosis which develops due to formate accumulation, provides a favorable pH environment for the enzyme to act. It has been reported in our previous published work [18]. Minimum optimal pH of formate dehydrogenase enzyme is 7.5 [19].

2. Materials and methods

2.1. Reagents

mPEG-SPA (mol.weight 5000) was from Fluka chemicals. Formate dehydrogenase (10.5 u/mg protein) from *C. boidinii*, Sephacryl 300 HR, and DEAE Sepharose FF were from Sigma-Aldrich Co, St. Louis, MO. Rabbit Anti-Rat IgG HRP conjugated secondary antibody was from Bangalore Genei, India. All other reagents were of analytical grade. Formate dehydrogenase enzyme has a molecular weight of 74 Kda [19]. The enzyme exists as a homodimer of 42 kDa subunits each possessing an independent active centre, and has no apparent metal-ion requirement [20].

2.2. Animals

Male Wistar rats (150–170 g) were used. Animals were maintained in a clean rodent room. They were housed 2–3 per cage in cages that were fitted with stainless-steel wire-mesh bottoms. They were maintained at a temperature of 28±1 °C, and under a daily photoperiod of 12-h light/dark cycle. The animals were fed with pellet diet (Hindustan Lever Ltd., Mumbai, India) and tap water ad libitum. The animals were handled according to the Committee for the Purpose of Control

and Supervision of Experiments on Animals (CPCSEA), Govt. of India. The experimentation protocol was reviewed and approved by Institutional Ethical committee.

2.3. PEGylation reaction

PEG reagents were dissolved in 20 mmol/l sodium phosphate buffer (pH 8.3) at concentration between 10 and 40 mmol/l. Initial reactions were carried out at molar ratios of PEG to protein between 40:1, 80:1 and 120:1 ratio to determine the optimal conjugation conditions and monitored on SDS-PAGE. The ratio of 40:1 was then used for all subsequent reactions.

PEGylation reactions were carried out in reaction buffer (0.2 mol/l sodium phosphate buffer, pH 8.3), at 20 °C for 30 min. The reaction buffer also contains 10 mmol/l sodium formate which is the substrate of the enzyme. The reactions were stopped with one-tenth volume of stop buffer (1 mol/l sodium phosphate buffer, pH 6.5) at 0 °C [21]. PEG–FD was then concentrated with Amicon Ultra-4 (10 kDa cut off) centrifugal filter unit.

2.4. Purification of PEG–FD

2.4.1. Sephacryl S-300 HR

Unreacted PEG was removed by size-exclusion chromatography [21]. Size exclusion column (30 cm×1 cm diameter) was packed with pre swollen Sephacryl S-300 HR (75–125 µm) micro beads purchased from Sigma Chemicals Pvt. Ltd, USA. Five milliliters of PEGylation products (1 mg/ml) were loaded on the column which was equilibrated and eluted with 0.15 mol/l sodium chloride in 10 mmol/l sodium phosphate (pH 7.4) at a flow rate of 1 ml/min.

2.4.2. DEAE Sepharose FF

The un-PEGylated FD was separated from PEGylated FD by DEAE Sepharose FF column chromatography [21]. Anion exchange column (30 cm×1 cm diameter) was prepared by using the DEAE Sepharose micro beads (25–45 µm) from Sigma Chemicals Pvt. Ltd. The column was equilibrated with equilibration buffer (50 mmol/l NaCl in 10 mmol/l sodium phosphate pH 7.4) at 1 ml/min flow rate.

2.4.3. Enzyme activity of PEG–FD

PEG–FD activity was measured in a mixture of 150 µmol potassium phosphate buffer pH 7.5, 5 µmol NAD, 500 µmol formate and limiting amounts of enzyme in a total volume of 3 ml. The increase in absorbance at 340 nm was read. [19]. One unit of enzyme was defined as the amount of enzyme that catalyzes the conversion of one micromole of NAD to NADH per minute at pH 7.5 at 37°C.

2.4.4. Serum half life of PEG–FD

In vivo serum clearance rates for both native FD and PEG–FD (40:1) were analyzed in 2 groups of albino rats, each consists of five rats (180–200 g). Purified PEG–FD or native FD (150 units) was injected into the left jugular vein. Blood was collected from the right jugular vein at the following time points namely before injection, 5, 15, 30, 45 min, 1, 2, 4, and 8 h after injection. Enzyme

activity was measured as described above. The half life of PEG–FD and that of native FD were calculated (21).

2.4.5. Immunization protocol

Male albino rats in groups of five, were injected with Native FD or PEG–FD (40:1) diluted in filter sterilized PBS buffer, intravenously (tail vein) on days 0, 8 and 15 with 0.1 ml (20 µg of protein) of the solution and bled from the tail vein on days 14 and 21 [22]. After coagulation for 1 h at room temperature, the blood samples were centrifuged for 20 min at 2000×g, at 4 °C to obtain sera. The sera were stored at –20 °C for further analysis.

2.4.6. Immunogenicity analysis by ELISA

For the quantification of serum antibodies (IgG) specific for FD and PEG–FD (40:1), 96-wells micro titer plates were coated overnight at 4 °C with 100 µl/well of a 5 µg/ml solution of FD or PEG–FD in carbonate buffer, pH 9.6 [23]. The plates were washed three times with water containing 0.4% Tween 20. This was followed by the addition of 100 µl/well phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 0.02% Tween 20. After 1 h incubation at 37 °C, the plates were washed and serial dilutions of rat serum in PBS/BSA-Tween 20 were added to the wells and incubated for 1 h at 37 °C. After washing, 100 µl/well peroxidase conjugated rabbit anti-rat IgG (H+L) in PBS/BSA-Tween 20 was added. After 1 h incubation at 37 °C, the plates were rewashed and enzyme substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB/H₂O₂, Bangalore Genei, India) (100 µl/well) was added. The plates were developed at room temperature for 15 min. Finally 100 µl/well of 2 mol/l H₂SO₄ was added. Optical densities were read spectrophotometrically at 450 nm with an ELISA plate reader (Labsystems Mutiscan MS, ver 8).

A pre immune sera pool was prepared by taking blood from the test animals before sensitizing them with concerned enzyme preparation. It was used as negative control. The average extinction in negative control wells provided a value which was taken as reference value to determine the titer in the test sera. Each test serum was titrated starting at 1: 10 dilution and highest serum dilution giving extinction higher than the reference value was taken as titer [23]. All analyses were performed in duplicate. Positive and negative control samples were incorporated for each 96-wells plate.

2.5. Allergenicity analysis

2.5.1. Passive cutaneous anaphylaxis

Five male wistar albino rats were immunized intravenously with 0.1 mg/kg of either native FD or PEG–FD. Seven days later, another dose of same amount of protein was given and bleeding of the animal was done on 14th day through tail vein. Sera samples were pooled on an experimental group basis and stored at –20 °C till further analysis.

Enzyme specific IgE titer was known by homologous passive cutaneous anaphylaxis test [23–25]. Four naive (untreated) Wistar albino rats were utilized per sample. The rats were shaven on the abdomen and injected intradermally with 0.1 ml of the test sera in serial dilutions, followed by 64 h later with an intravenous infusion of 1 ml of a 1:1 mixture of a

solution of native FD or PEG–FD (5 mg/ml sterile Saline) and a solution of Evans blue (0.5%). After 20–30 min, the animals were examined for positive responses and the diameter of cutaneous reactions was measured. A positive (IgE) response was recorded if the challenge resulted in a >5 mm blue lesion in the skin in the majority of recipient animals. IgE titer was recorded as the highest dilution of serum resulting in a positive PCA reaction. Positive and negative control sera were analysed simultaneously on each animal used for the PCA tests.

2.5.2. Active systemic anaphylaxis test

Five rats per group were used for tests at doses of 0.1 and 1.0 mg/kg of test materials which were given intravenously on days 1, 3 and 5 respectively. Two weeks later, a systemic anaphylaxis reaction was induced by injecting the test materials intravenously into the animals at a dose of 4.0 mg/kg, and the reactions were monitored for 30 min and scored [9].

2.5.3. In vivo efficacy of PEG–FD in eliminating formate

In vivo efficacy of PEG–FD (40:1) or native FD was comparatively evaluated by single intravenous administration of PEG–FD or native FD in folate deficient methanol intoxicated albino rats along with Carbicarb (Carb) buffer infusion. Male albino rats were divided into eight groups namely Saline control, Enzyme control, MeOH control, FDD (Folate Deficient Diet) control, FDD–MeOH, FDD–MeOH–Carb, FDD–MeOH–Carb–Native FD, FDD–MeOH–Carb–PEG FD. Each consists of 6 rats.

Out of 8 groups, 5 were made folate deficient by providing folate deficient diet (FDD) for 45 days [26] and water ad libitum. Folate deficiency was further confirmed by estimating urinary excretion of formamino glutamic acid (FIGLU) on the 45th day [27].

Carbicarb (Carb) solution was prepared in sterile saline [15]. For treatment, methanol (MeOH) was diluted in sterile saline (20% w/v solution) and administered intraperitoneally. FDD–MeOH control, FDD–MeOH–Carb, FDD–MeOH–Carb–Native FD, FDD–MeOH–Carb–PEG FD and MeOH control groups were administered with methanol diluted in saline (3 g/kg) (i.p.). Saline control, Enzyme control and FDD control groups received equivalent volume of saline (i.p.). After 12 h, FDD–MeOH–Carb, FDD–MeOH–Carb–Native FD and FDD–MeOH–Carb–PEG FD groups were infused (i.v.) with Carbicarb solution (2 ml/kg) through right jugular vein over a period of 2 min. After 3 min, FDD–MeOH–Carb–Native FD, FDD–MeOH–Carb–PEG FD and enzyme control group were administered with 200 µl enzyme in saline (200 units of PEG–FD or native FD enzyme in saline) (i.v.) through right jugular vein. All other groups received equal amount of saline (i.v.). After intravenous infusion of enzyme, blood samples were collected for 1 h, at every 15-min time points from the left jugular vein. Prior to intravenous infusion of Carbicarb and Enzyme or saline, one blood sample was taken which was considered as 0 time value. Blood methanol and formate level were quantified.

2.5.4. Blood methanol measurement

One microliter of plasma was deproteinized with equal volume of acetonitrile and centrifuged at 5000×g for 7 min.

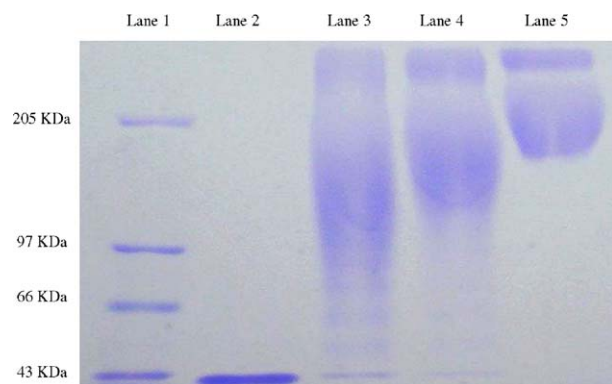


Fig. 1. SDS-PAGE (7.5%) of Native and PEGylated Formate dehydrogenase at different PEG/FD ratios. Lane 1 M.W. markers (Broad range); Lane 2, Native Formate dehydrogenase (FD); Lane 3, PEG–FD at 40:1 ratio; Lane 4, PEG–FD at 80:1 ratio; Lane 5, PEG–FD at 120:1 ratio. The migration rates of PEG–FD molecules were much slower than those of native FD.

Supernatant was analyzed by HPLC refractive index detector system (Shimadzu RID 10A) equipped with a Rezex ROA–Organic acid column (300×7.5 mm i.d; Phenomenex) [28] and security guard cartridge (AJO 4490, Phenomenex). Column oven was used to maintain the temperature at 60 °C. The mobile phase was 0.026 mol/l H₂SO₄ [29,30,8]. Methanol was used as an external standard. Recovery studies of methanol from blood were done and it was found to be 93–96%. Linearity for methanol was 5–500 mg/dl. The detector sensitivity for methanol was 2 mg/dl and the reproducibility was >94%.

2.5.5. Blood formate measurement

Protein free supernatants were prepared from the whole blood by the sequential addition of 7.5% ZnSO₄ and 0.4 mol/l NaOH. formate analysis was performed by the coupled formate dehydrogenase–diaphorase enzymatic method [31]. The fluorescence of each sample was determined by Hitachi 650-10 M fluorescence spectrophotometer with an excitation wavelength at 565 nm and emission wavelength of 590 nm.

2.5.6. Statistical analysis

All the data were statistically analyzed using one-way analysis variance (ANOVA) followed by Tukey's multiple comparison test. Student's *t*-test was used when one comparison was made between 2 groups. A *P*<0.05 was considered

Table 1
Systemic anaphylaxis reaction

Test substance	Dose (mg/kg) (i.v.) given for sensitization	No. of rats with following severity of reaction				
		±	+	++	+++	++++
Native FD	0.1	1	1	2	1	
	1.0			1	2	2
PEG–FD	0.1	5				
	1.0	4	1			

The dose to induce anaphylaxis by each test substance was 4.0 mg/kg. Severity of anaphylaxis: '±' no symptom; '+' scratching; '++' tremor; '+++ dyspnea and/or cough; '++++' death.

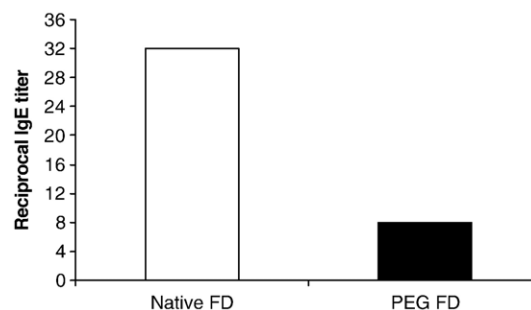


Fig. 2. Passive cutaneous anaphylaxis test for native FD and PEG–FD. Serum samples were pooled on an experimental group basis and IgE titer of the pooled sera was assayed by PCA testing. Antibody titer recorded as the highest dilution of serum resulting in a positive PCA reaction >5 mm blue lesion in the skin of the majority of recipient animals.

significant. The results shown are mean±S.D. Software SPSS (ver 10) was used for statistical analysis.

3. Results

3.1. Purification of PEG–FD

After the conjugation reaction of FD with mPEG-SPA 5000, Sephacryl S-300 HR column was used to remove the excess free PEG. The PEG–FD and native FD were eluted in the exclusion volume. The fractions containing PEG–FD and native FD were eluted at approximately 25–35 ml. Enzyme elution was identified by absorbance at 280 nm and by activity assay. Free mPEG-SPA eluted in later fractions.

Fractions containing PEG–FD and native formate dehydrogenase were loaded into DEAE Sepharose FF column chromatography. PEG–FD was eluted with 50 mmol/l NaCl in 10 mmol/l sodium phosphate, pH 7.4 and the native FD was eluted with 140 mmol/l NaCl in 10 mmol/l sodium phosphate, pH 7.4. The fractions containing PEG–FD were eluted at approximately 30–40 ml. They were identified by absorbance at 280 nm and activity assay. Pegylation was confirmed by electrophoresis on SDS-PAGE.

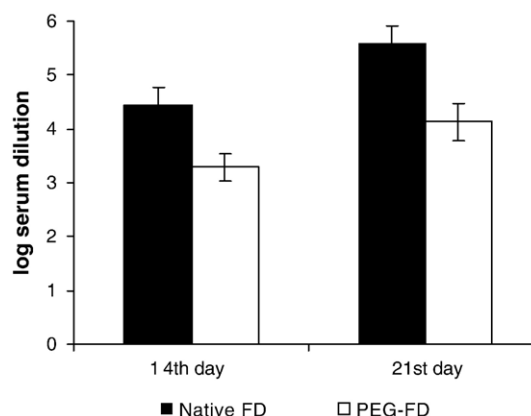


Fig. 3. Immunogenicity (IgG titer) of Native FD and PEGylated FD at 14th day and 21st day after intravenous infusion of 0.1 ml of test substance (20 µg of Native FD or PEG FD) on 0, 8th and 15th day.

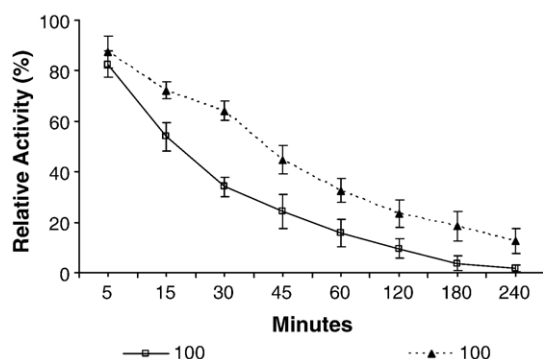


Fig. 4. Plasma half life of Native FD and PEG-FD. After intravenous infusion of enzyme (150 units) enzyme activity was calculated at different time points. Relative to the activity at 0-min time point, percentage of activity at all other points were calculated and plotted. Activity at 0-min time point was taken as 100%.

3.2. Confirmation of PEGylation

SDS PAGE analysis was carried out using 7.5% polyacrylamide gel [32] and stained with Coomassie Brilliant Blue R-250. Bangalore Genei Molecular weight standards, Broad range, was utilized. SDS-PAGE analysis (Fig. 1) demonstrated PEGylation of native FD, at different PEG/FD ratios namely 40:1, 80:1, and 120:1. The migration rates of PEG–FD molecules (Lanes 3, 4, 5) were much slower than those of native FD (Lane 2). Conjugation of PEG molecules to the protein led to increase in the molecular weight. In the Lane 3–5 sharp bands were not seen because of variance in the number of PEG molecules attached to the polypeptide chain of the enzyme. Similar observations have already been reported [21]. In the Lanes 3 and 4, apart from the PEG–FD, at the leading end of the gel, a small amount of unmodified FD can also be seen. When PEG/FD ratio increased to 120:1 almost all the FD molecules were converted into PEG–FD (Lane 5). The PEG–FD synthesized at the ratio of 40:1, 80:1, 120:1 respectively had activity of 60–70%, 20–25% and 1–5% of that of native enzyme.

3.3. Allergenicity

The systemic induction of anaphylaxis by native FD and PEG–FD was examined (Table 1). Rats treated with native FD exhibited strong reactions such as scratch, tremor, dyspnea and death while rats treated with PEG–FD showed no symptoms or only a very weak reaction. Significant fall in the IgE titer was observed (Fig. 2) in the PEG–FD treated group when compared to native FD treated group.

3.4. Immunogenicity

Serum IgG titer for native, PEG–FD (40:1) was estimated. Significant decrease in the IgG titer was observed for PEG–FD both in the 14th day and 21st day, in relation to native FD (Fig. 3).

3.5. Serum half life of PEG–FD

The $t_{1/2}$ in serum of PEG–FD after iv injection in rats was approximately 33 min compared to approximately 13 min of native FD. The results demonstrated that the serum half life of PEG–FD was increased (Fig. 4).

3.6. In vivo efficacy of PEG–FD

Blood methanol level, 12 h after intraperitoneal administration, was 249.6 ± 3.48 mmol/l, in the MeOH control animals. Blood methanol was estimated to correlate with the blood formate level. Significant formate accumulation was found in the FDD–MeOH control and in the FDD–MeOH–Carb groups when compared to the methanol control.

Significant elimination of formate was observed both in the FDD–MeOH–Carb–Native FD, FDD–MeOH–Carb–PEG FD groups at 15-, 30-, 45- and 60-min time points when compared to FDD–MeOH–Carb (Fig. 5). Elimination of formate was more in the FDD–MeOH–Carb–PEG FD when compared to FDD–MeOH–Carb–Native FD at 30-, 45- and 60-min time points.

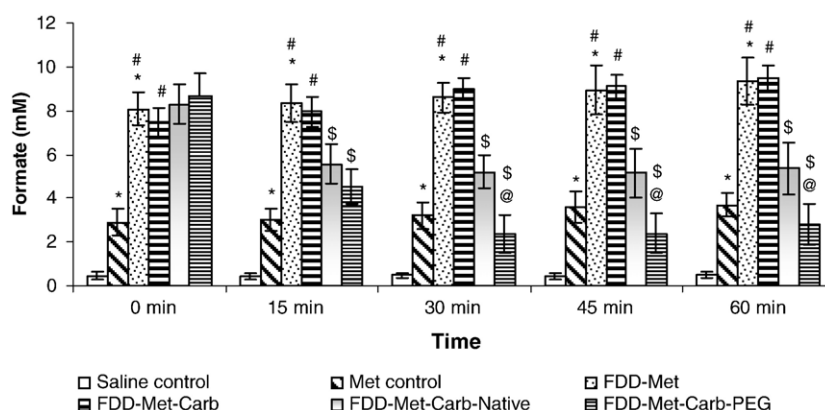


Fig. 5. formate level at different time points. Zeromin value was noted 12 h after methanol exposure, before Carbicarb/enzyme infusion. “**” significance at $P < 0.05$ with Saline control. “#” significance at $P < 0.05$ with MeOH control. “\$” significance at $P < 0.05$ with FDD–MeOH–Carb. “@” significance at $P < 0.05$ with FDD–MeOH–Carb–Native FD. Saline control was compared with MeOH control and FDD–MeOH control. MeOH control was compared with FDD–MeOH control and with FDD–MeOH–Carb. FDD–MeOH–Carb group was compared with FDD–MeOH–Carb–Native FD and with FDD–MeOH–Carb–PEG FD group. FDD–MeOH–Carb–PEG FD was compared with FDD–MeOH–Carb–Native FD group. Data shown are mean $\pm$ S.D.

Insignificant changes were observed in the FDD control and Enzyme control with respect to the saline control (data not shown).

4. Discussion

Attachment of PEG to proteins often takes place on the amino (ϵ -NH₂) group of lysine residues or at the N-terminus, since, the reaction occurs readily under mild coupling conditions [33]. There are 31 lysine residues in the primary structure of NAD dependent formate dehydrogenase isolated from the *C. boidinii* (UniProtKB/TrEMBL Database Primary Accession No. 013437). Active site characterization of formate dehydrogenase did not reveal the presence of lysine residues [35]. Therefore, it is possible to couple several mPEG moieties to FD so that, although its molecular weight is altered, its enzymatic activity is not substantially affected. A number of reactive esters of PEG are available to be selectively conjugated to the ϵ -NH₂ groups of lysine residues, for the formation of stable amide bonds in buffered solutions [34]. Linear mPEG-SPA 5000 was utilized for modification of enzymes such as Methioninase, Carboxy peptidase A for cancer therapy and tumor targeting respectively [21,33].

The coupling reaction was carried out under basic conditions (pH 8.0) so that the attachment of mPEG to FD at other residues other than lysine could be minimized [33]. The reaction buffer also contains sodium formate which is the substrate of the enzyme, added to protect the active site of the enzyme from pegylation. By preparing the activated mPEG solution at low temperature prior to PEGylation, one could minimize the loss of mPEG activity due to hydrolysis.

Improvement of pharmacokinetic, immunological and stability properties of asparaginase due to pegylation have been reported [10]. In our study, increased plasma half life, reduced immunogenicity and reduced allergenicity were observed for PEG–FD when compared to native FD. The decreased antigenicity of the modified proteins can be thought as a consequence of masking the protein antigenic sites by the polymer modification. More over, a hindering effect due to the polymer cloud around the protein structure can, to some extent, avoid the Ag/Ab interactions [10]. Fall in the immunogenicity might have led to decrease in the neutralizing antibodies which can cause inactivation or inhibition of enzyme.

Elimination of formate at 30-, 45-, 60-time points, was more in the PEG–FD treated group than in the native FD. This can be explained by increased plasma half life of PEG–FD, which was observed in this study.

Decrease in the immunogenicity and allergenicity of formate dehydrogenase may be helpful clinically when situation warrants repeated dosing of the enzyme. PEGylated formate dehydrogenase, because of its comparatively better efficacy to native FD, may be more useful in detoxification of formate in methanol poisoning. Reductions in immunogenicity and allergenicity have been reported in this study, but, immunotoxicity due to other mechanisms has not been completely ruled out, which needs further study.

When methanol poison victim reports with high formate level, current clinical choices of treatment for elimination of formate, are hemodialysis and infusion of active form of folate, folinic acid

[36]. Folinic acid administration increases folate reserve and indirectly facilitates metabolism of formate. Rapid elimination of formate is possible with hemodialysis, but, it is not readily available in all the places in developing countries. Whereas, PEGylated formate dehydrogenase enzyme can be kept in all the places in lyophilized form and bolus intravenous infusion of this enzyme may be useful in eliminating the formate rapidly.

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