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# Comparative biodistribution and safety profiling of olmesartan medoxomil *oil-in-water* oral nanoemulsion

Bapi Gorain [a, b, *], Hira Choudhury [a, c], Rakesh Kumar Tekade [d], Saumen Karan [e], P. Jaisankar [f], Tapan Kumar Pal [a, **]

[a] *Bioequivalence Study Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India*
[b] *Faculty of Pharmacy, Lincoln University College, Kuala Lumpur, Malaysia*
[c] *Department of Pharmaceutical Technology, School of Pharmacy, International Medical University, Malaysia*
[d] *National Institute of Pharmaceutical Education and Research (NIPER) – Ahmedabad, Department of Pharmaceutics, Opposite Air Force Station Palaj-Basan Road, Gandhinagar, Gujarat 382355, India*
[e] *Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India*
[f] *Department of Chemistry, Indian Institute of Chemical Biology, Jadavpur, Kolkata, India*

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ABSTRACT

Poor aqueous solubility and unfavourable de-esterification of olmesartan medoxomil (a selective angiotensin II receptor blocker), results in low oral bioavailability of less than 26%. Improvement of oral bioavailability with prolonged pharmacodynamics activity of olmesartan in Wistar rats had been approached by nanoemulsification strategy in our previous article [*Colloid Surface B, 115, 2014: 286*]. In continuation to that work, we herewith report the biodistribution behaviour and 28-day repeated dose sub-chronic toxicity of olmesartan medoxomil nanoemulsion in Wistar rats following oral administration. The levels of olmesartan in collected biological samples were estimated using our validated LC-MS/MS technique. Our biodistribution study showed significantly higher brain concentrations of olmesartan (0.290 ± 0.089 μg/mL, 0.333 ± 0.071 μg/mL and 0.217 ± 0.062 μg/mL at 0.5, 2.0 and 8.0 h post dosing, respectively) when administered orally as nanoemulsion formulation as compared to the aqueous suspension. In addition, the olmesartan nanoemulsion was found to be safe and non-toxic, as it neither produced any lethality nor remarkable haematological, biochemical and structural adverse effects as observed during the 28-days sub-chronic toxicity studies in experimental Wistar rats. It is herewith envisaged that the developed nanoemulsion formulation approach for the delivery of olmesartan medoxomil via oral route can further be explored in memory dysfunction and brain ischemia, for better brain penetration and improved clinical application in stroke patients.



## 1. Introduction

Lipophilic nature of most of the new chemical entities entails limitations in aqueous solubility leading to poor oral bioavailability (Nasr et al., 2016). Olmesartan, one of the newer antihypertensive agents, acts by selective blockade of AT1 - subtype angiotensin II receptor (Gorain et al., 2013). However, its poor aqueous solubility and unrestrained enzymatic conversion of olmesartan medoxomil in the gastric fluid to its insoluble parent molecule further restricts its oral bioavailability to only 26% (Nasr et al., 2016). Encapsulation of the lipophilic drugs inside nano-sized oily core and the stabilization of this oily reservoir using an appropriately selected surfactant layer leads to the formation of a stable nanoformulation that resolves its solubility as well as biopharmaceutical issue. With this motivation, several approaches have been explored for solubility enhancement and oral delivery of olmesartan medoxomil, viz: solid dispersion (Sathali and Jayalakshmi, 2013), nano-suspension (Attari et al., 2016), complexation (Gera et al., 2015) and self-microemulsifying drug delivery (Nasr et al., 2016) to name the few.

In this context, the investigations on the development of oral nanoemulsion delivery system for the biopharmaceutics

* Corresponding author. Lincoln University College, 47301, Petaling Jaya, Kuala Lumpur, Malaysia.
** Corresponding author. Department of Pharmaceutical Technology, Jadavpur University, Kolkata, 700 032, India.
*E-mail addresses:* bapi@lincoln.edu.my, bapi.gn@gmail.com (B. Gorain), proftkpal@gmail.com (T.K. Pal).

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classification system (BCS) class II and class IV drugs is one of the focused area of current research (Choudhury et al., 2017; Devalapally et al., 2015; Gue et al, 2016). It has been widely reported that the encapsulation of BCS class II and class IV drugs in the core of nanoemulsion enhances their solubility, increases interfacial area, minimizes irritation due to rapid biodistribution, reduces their interfacial tension (Patel and Joshi, 2012; Jaiswal et al., 2015). This strategy also extends the gastric residence time, stimulates intestinal lymphatic transport pathway, alters intestinal permeability, decreases drug efflux and diminishes its metabolism (Choudhury et al., 2014; Garcıa-Celma et al., 2016).

In our most recent publication, a nano-formulation approach was reported for the improvement of pharmacokinetic and therapeutic activity of olmesartan medoxomil. Our developed nanoemulsion approach enhanced the oral bioavailability of olmesartan by 2.8-fold and prolonged the pharmacodynamic activity in hypertensive animal model. The thermodynamically stable nanoemulsion of olmesartan medoxomil comprised of nano-sized spherical oil droplets (<50 nm) and showed superior permeability while assessed in Caco-2 cell monolayer. The formulation of olmesartan was accomplished using biocompatible components, viz: Lipoid purified soybean oil 700 (8%, *v/v*) and sefsol 218 (propylene glycol caprylate; 8%, *v/v*) as oil phase and solutol HS 15 (18%, *v/v*) as non-ionic surfactant (Gorain et al., 2014). The components for the preparation of nanoemulsion formulation were judiciously selected by considering their aesthetic value, non-irritancy, cellular non-sensitization as well as Generally Regarded as Safe (GRAS) tag (Baboota et al., 2011; DeMerlis et al., 2009). In continuation of our previous work, we hereby report the organ biodistribution and 28-days sub-chronic toxicity of olmesartan nanoemulsion following its oral administration in Wistar rats. This reports sheds clearer information regarding the accumulation tendency of the drug in different organs. In similar line, the repeated dose toxicity studies can identify the toxicity of the test substance after its continuous exposure (OECD, 2008). It helps to extrapolate the safe dose of the investigating formulation for clinical application (Nuffield Council on Bioethics, 2005). Hence, the organ distribution study and toxicity profile of the optimized nanoemulsion of olmesartan medoxomil were evaluated in this investigation. The obtained data was compared with the olmesartan medoxomil suspension to gather comprehensive preclinical investigational information with regards to changes in hematological, biochemical parameters and histopathological characteristics.

## 2. Materials and methods

### 2.1. Chemicals

Working standard of olmesartan medoxomil (purity, >99%) was gifted from Burgeon Pharmaceuticals, Chennai, India. The internal standard (IS), telmisartan (purity, >99%), was obtained from Akums Drugs & Pharmaceuticals Ltd., New Delhi, India. It has been observed that the compounds from same chemical class behave in similar manner in mass spectra and also nullify human error without interfering its analysis (Choudhury et al., 2016). As telmisartan belongs to the same chemical class of olmesartan, synthetic imidazole derivative, it has been integrated in the current analysis as IS. HPLC grade chemicals and reagents used in this current experiment were purchased from Merck Pvt. Ltd (Mumbai, India). Further, HPLC grade water (resistivity of 18.2 MW cm) generated from Milli Q water purification system (Elix, Milli Q A10 Academic, Molsheim, France) was used throughout the analytical procedure.

### 2.2. Preparation of olmesartan medoxomil nanoemulsion

An *oil-in-water* nanoemulsion containing olmesartan medoxomil was prepared by using spontaneous aqueous titration method as reported by us in our previous publication (Gorain et al., 2014). The developed formulation was processed through several processes to optimize the morphology, mean particle size, surface zeta potential, viscosity, refractive index, electrical conductivity and *in vitro* release of the formulation (Gorain et al., 2014).

### 2.3. Animal handling and care

A sub-chronic repeated dose (28 days) toxicity study in Wistar rats was conducted according to the OECD 407 guideline (OECD, 2008). The maintenance of animal handling and care was performed under the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA). Male Wistar rats (weight: 120–130 g) were selected for the *in vivo* biodistribution study, whereas, for the toxicological evaluation animals of both sexes were designated within the same weight range. Precautions were taken to ensure that the selected female animals were nulliparous and non-pregnant. The animals were acclimatized under the standard controlled conditions, (temperature, 25 ± 5 °C; relative humidity, 55± 10%) with light and dark cycle of 14 h and 10 h, respectively. All the projected research animal activities and animal testing were performed after complete review and approval of the proposal by the Animal Ethics Committee, Bioequivalence Study Centre, Jadavpur University, Kolkata. A period of 7–10 days was provided to the animals to acclimatize under stipulated laboratory conditions prior to the initiation of experimentation. Throughout the experimentation period, animals were housed in polypropylene cages provided with standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum* throughout the study.

### 2.4. Bio-distribution study

#### 2.4.1. Drug administration and sampling plan

Bio-distribution study of the developed nanoemulsion was performed to track the distribution of olmesartan in different organs in the experimental animals according to Yin et al. (2012). In this experimentation, male rats were fasted for 10 h, with free access to water. Animals were divided into two groups with 12 animals in each to assure parallel study design. Animals were administered with developed nanoemulsion and 0.25% carboxy methyl cellulose (CMC) suspension of olmesartan medoxomil by oral route at a dose of 10 mg/kg and 30 mg/kg body weight, respectively. A decreased dose of nanoemulsion was chosen based on outcome of our previous pharmacokinetic study as resulted by the increased bioavailability of olmesartan (2.8 fold) when administered through oral nanoemulsion delivery system as compared to the suspension (Gorain et al., 2014). A total of four experimental rats from each group were sacrificed by cervical dislocation at 0.5, 2.0 and 8.0 h time points following the oral administration of formulations. After this, liver, lung, kidney and brain tissue were carefully isolated.

The time points were judiciously selected to justify the estimated exposure time, based on our previous published findings on pharmacokinetic profile (Gorain et al., 2014). The peak plasma concentration was achieved in 0.5 h followed by its elimination phase. Therefore, in our present experimental design, we collected the organ samples (liver, lung, kidney and brain) during peak plasma drug level (i.e., during absorption phase) followed by distribution (2.0 h) and elimination phase (8.0 h) of the drug from the system. Organ samples were then rinsed with physiological saline

solution (0.9% *w/v* NaCl), dried and stored in biofreezer (at −20°C) until analysis of the collected organs for the presence of olmesartan by liquid chromatography–tandem mass spectrometry.

*2.4.2. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis and chromatographic conditions*

A Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) comprising of LC-20AD pump, DGU-20A3 prominence degasser, SIL-20AC autosampler, CTO-10ASVP column oven (Shimadzu, Kyoto, Japan) and controller (CBM20A Lite) was used to analyze the organ samples for olmesartan concentration Mass spectrometric detection was performed on an API 2000 triple quadrupole mass spectrometry (AB Sciex Instruments, Toronto, Canada) equipped with an electrospray ionization source operating with a Turboionspray™ interface at 400 °C. Analytical data processing was performed by Analyst software (version 1.5).

The HPLC separation was achieved on an Agilent $C_{18}$ column (75 × 4.6 mm, 3.5 μm; Agilent, USA) using 30 μL injection volume by anisocratic mobile phase consisting of 10 mM ammonium acetate and acetonitrile (10:90, *v/v*) at a flow rate of 0.5 mL/min. The MS–MS detection was achieved in negative ion modes for the analyte and internal standard (IS; Telmisartan) using multiple reaction monitoring with 200 ms dwell time with transition pair of m/z 445.1/149.2 for olmesartan and m/z 513.3/287.0 for IS with the ion source parameters, viz. curtain gas, GS1 gas and GS2 gas, were set at 15, 45 and 50 L/min, respectively, whereas the CAD gas was set at 12 L/min. Compound dependent chromatographic parameters are mentioned in Table 1.

During analysis, frozen organ samples were thawed at room temperature, weighed and homogenized at 10,000 rpm for 5 min (Tissue Homogenizer, Hahntech Corporation, South Korea) with ice-cold physiological saline solution at a ratio 1:2 (g:mL) followed by sonication (Ultrasonic Homogenizer, Takashi Electric Co. Ltd., Japan) on wet ice for 1 min.

Our in-lab developed and validated LC-MS/MS method following US FDA guideline for bioanalytical method was used for the estimation of olmesartan concentration in plasma and organ samples, wherein, 0.010 and 5.0 μg/mL were considered as lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) for different organ samples, respectively. Extraction of analyte from the homogenized organ samples were performed using 4 mL of extracting solvent, ethyl acetate, by hand mixing for a duration of 12 min. Centrifugation process at 5000 rpm was followed for 10 min, thereafter 3.2 mL supernatant was separated and dried under inert atmosphere. Residue was reconstituted with 200 μL mobile phase and 30 μL of the reconstituted sample was injected into the LC-MS/MS for quantification. The response was linear in the concentration range 0.010–5.00 μg/mL, with a coefficient of determination ($r^2$)>0.99 for each organ samples (Gorain et al., 2014).

*2.5. Twenty-eight-day sub-chronic oral toxicity in Wistar rats*

*2.5.1. Randomization, numbering and grouping of animals*

The 28-day oral sub-chronic toxicity study of the olmesartan medoxomil nanoemulsion compared to olmesartan medoxomil suspension was evaluated following the recommendations of OECD (2008) and was conducted in compliance with FDA Good Laboratory Practice Regulations (Part 58 of 21 CFR). Briefly, eighty-four healthy Wistar rats were selected for the sub-chronic toxicity study consisting thirty-six rats in each major evaluation groups and twelve rats in control group. Each major group was further subdivided into three (twelve in each), composed of the low, medium and high dose treatment subjects (discussed in detail under 'Dosing of animals'). Finally, these subgroups were divided into two groups consists of six males and six females. Detailed allocation of the animals is represented in supplementary Table S1.

*2.5.2. Dosing regimen*

The dose levels for olmesartan medoxomil formulation in this current toxicity study were carefully chosen according to the reported sub-chronic oral toxicity study in experimental rats (Sengupta et al., 2012). Therefore, a dose of 150 mg/kg/day, 75 mg/kg/day and 40 mg/kg/day were selected as high, medium and low doses, respectively for the olmesartan delivered as 0.25% carboxymethyl cellulose suspension, whereas, 60 mg/kg/day, 30 mg/kg/day and 15 mg/kg/day dosages were selected for the optimized nanoemulsion formulation of olmesartan medoxomil. Notably, the dose of nanoemulsion was selected on the basis of our previous finding on the pharmacokinetic parameters of nanoemulsion (Gorain et al., 2014), and the reported dose dependent toxicity data (Sengupta et al., 2012).

Animals in the control group were treated with normal saline (1 mL/animal). In this context, it is important to mention that the calculated volume of nanoemulsion was larger than the usual recommended volume (<20 mL/kg) for orally administered rats (Turner et al., 2011) and for the said reason we adopted a divided dosing protocol to administer the daily dose of nanoemulsion. Thus, we administered the experimental animals with the calculated volume of nanoemulsion in two divided doses at a gap of 2 h.

*2.5.3. Clinical observation and body weight trends*

Detailed clinical observations for any clinically abnormal signs were performed to all the experimental animals throughout the study period. Signs of toxicity, unusual behaviour, irritability, morbidity, and mortality were noted every day to correlate the observed changes in the experimental animals. Body weight of the individual experimental rat was recorded before starting the experiment, and thereof every 7th day during the course of study. Mean body weight of animals at different time points were finally compared with the change in body weight. It is imperative to note that the individual dose of animals were adjusted for the body weight to maintain the same target dose level in all the animals.

*2.5.4. Food consumption behaviour*

For evaluation of food consumption behaviour, a group of six Wistar rats were carefully monitored for their food consumption habits before and throughout the treatment period. Amount of daily food consumption was calculated by using the following equation:

$$\text{Daily food consumption}\left(\frac{\frac{\text{g}}{\text{rat}}}{\text{day}}\right) = \frac{\textit{Food supplied to the cage} - \textit{Food remnants in that cage}}{6}$$

[Animal present per cage were 6, therefore, the daily food consumption was divided by '6' to determine the food consumption by each rat].

*2.5.5. Clino-pathological analysis*

Blood from each animal was collected by retro-orbital venipuncture for pathological evaluation during completion of the toxicity study, i.e., on day 29, to correlate the findings clinically. Evaluation of blood samples for the haematological and biochemical parameters are thought to be essential markers to assess the health status of the animals, as well as human being. Following clino-pathological analysis were done for the validation of clinical and laboratory diagnosis.

**Table 1**
Chromatographic parameters of the analyte (olmesartan) and the IS (telmisartan) used during quantification in LC-MS/MS.

| Parameters | | Olmesartan | IS (Telmisartan) |
|---|---|---|---|
| Molecular weight | | 446.5 | 514.6 |
| Run time (min) | | 2.0 min | |
| Mass fragments | Q1 mass | 445.1 | 513.3 |
| | Q3 mass | 149.2 | 287.0 |
| Declustering potential (DP) | | −45 V | −50 V |
| Collision energy (CE) | | −45 eV | −45 eV |
| Entrance potential (EP) | | −10 V | −10 V |
| Collision cell exit potential (CXP) | | −10 V | −22 V |

*2.5.5.1. Haematological analysis.* Blood samples (0.5 mL) collected into potassium EDTA containing tubes were analyzed quickly for haematological parameters. The analysis was performed by Medonic CA-620 cell analyzer system (Boule Medical, Stockholm, Sweden) to measure red blood cell count, haemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, reticulocytes, total leukocyte count and differential counts (neutrophil, lymphocyte, monocyte and eosinophil).

*2.5.5.2. Serum biochemistry markers.* Measurement of biochemical marker level is an important aspect while targeting any specific toxicological event. Therefore, along with haematological analysis, separate blood samples (0.5 mL) were also collected in tubes devoid of anticoagulant for serum biochemistry analysis. Following coagulation of the blood at room temperature, the tubes were centrifuged at 3000 rpm for 10 min. Microlab-300 autoanalyzer (Merck Pvt. Ltd. Mumbai, India) was employed to estimate total protein, blood urea nitrogen, serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase and creatinine levels from the separated blood serum.

*2.5.5.3. Organ-body weight index.* Following collection of the blood samples for clino-pathological analysis, all the experimental animals were euthanized and the principal organs like liver, kidney, lung and stomach were removed. Immediately after collection, the organs were macroscopically analysed for any anatomical or pathological abnormalities. Collected organs were dried, weighed and calculated for organ-body index utilizing the following formula (Venkatasubbu et al., 2015).

$$Organ-body\ weight\ index\ (\%) = \frac{Wet\ organ\ weight}{Body\ weight} \times 100$$

*2.5.5.4. Histopathological analysis.* After determination of the organ-body weights, all the organs were fixed in a 10%, *v/v* solution of neutral buffered formalin (Venkatasubbu et al., 2015). Standard pathological procedure of defatting and dehydration of the organs was adopted following embedding in paraffin, and sectioned to 3–5 μm using microtone. Subsequently, staining of the tissue samples were performed by placing those sectioned tissues onto glass slides and by the use of hematoxylin–eosin. Sections were later evaluated under an optical microscope (scale 1:40). Pictures of the developed specimen slides were evaluated by an experienced pathologist keeping blinding to the treatment groups.

### 2.6. Statistical evaluation

For statistical significance, different experimental data were compared with the corresponding control data using one-way analysis of variance test (ANOVA) followed by the Dunnett's test. A $p$ value $< 0.05$ was set as statistical significance for all tests. All results were expressed as mean ± standard deviation (S.D.) for the indicated number of experiments.

## 3. Results and discussion

An oil-in-water (*o/w*) nanoemulsion of olmesartan medoxomil was developed by aqueous titration method using the blend of biocompatible Lipoid purified soybean oil 700 (8% *v/v*), sefsol 218 (8% *v/v*) and solutol HS 15 (18% *v/v*) by solubility determination of drug in oils/surfactants and in combinations thereof (Gorain et al., 2013). According to our published report on the developed *o/w* olmesartan medoxomil nanoemulsion, it is confirming that the developed formulation is thermodynamically stable and facilitating its delivery as well as enhancement of oral bioavailability upto 2.8 fold with prolong control to the hypertensive condition of experimentally induced hypertensive rats (Gorain et al., 2014). The characteristics of the optimized formulation containing 2 mg/mL equivalent olmesartan medoxomil were summarized in Table S2 (Gorain et al., 2014).

The organ distribution analysis as reported in this connecting manuscript will help the researchers in the allied field to further understand the concept of tendency of tissue distribution pattern of olmesartan when delivered through nanoemulsion formulation.

### 3.1. Biodistribution analysis

It was anticipated that biodistribution following oral administration will provide the level of the drug in different tissues relative to the circulatory level (Griffin et al., 2016). Hence, tissue distribution study of olmesartan following oral administration of nanoemulsion formulation was performed in order to obtain a comprehensive relationship between the plasma concentration and level of drug in different organs. In this accord, so as to determine the traces of drug in the organ sample, we developed a rapid, simple and sensitive LC-MS/MS bioanalytical method for the estimation of olmesartan. In due course, the chromatographic conditions were optimized to achieve reasonable separation and reproducible peaks of olmesartan and internal standard (IS) from the bio-matrix without any interfering peaks from the endogenous compounds within the run time of analyte and IS.

Judicious selection of compound dependent and ion-source parameters (as depicted in Table 1) of the instrument lead to the generation of intense peaks for the product ions at *m/z* 149.2 and *m/z* 287.0 for olmesartan and IS, respectively. The Q1 and Q3 full scan spectra for the compound and IS are represented in Fig. 1. It can be evinced that the improved peaks of olmesartan and IS were achieved at 1.20 and 1.80 min, respectively, with the mobile phase composed of 10 mM ammonium acetate and acetonitrile (10:90 *v:v*) when delivered at a flow rate of 0.5 mL/min. The obtained chromatograms were devoid of any interfering substances with a total runtime of 2.0 min. Typical multiple reaction monitoring

(MRM) chromatograms of different brain matrixes following administration of olmesartan medoxomil nanoemulsion have been represented in Fig. 2.

Due course of analysis, maximum concentration of olmesartan was found in the liver (1.22 ± 0.136 μg/g of tissue or $1.02 \times 10^{-3}$ part of the delivered dose/g of liver tissue) followed by lungs (0.76 ± 0.250 μg/g of tissue or $6.3 \times 10^{-4}$ part of the delivered dose/g of lungs tissue), kidney (0.66 ± 0.104 μg/g of tissue or $5.5 \times 10^{-4}$ part of the delivered dose/g of kidney tissue) and brain (0.290 ± 0.088 μg/g of tissue or $2.4 \times 10^{-4}$ part of the delivered dose/g of brain tissue) at 0.5 h following the oral administration of the nanoemulsion. This trend of drug distribution in different organs remained constant at 2.0 h post dosing time point, but, notably the drug concentration in these organs progressively increased. Subsequent analysis of drug level in various organ samples at 8.0 h, the drug concentration in kidney was found to highest in the animal group received suspension of olmesartan medoxomil (0.32 ± 0.821 μg/g of tissue or $8.9 \times 10^{-5}$ part of the delivered dose/g of kidney tissue) and nanoemulsion of olmesartan medoxomil (0.78 ± 1.812 μg/g of tissue or $6.5 \times 10^{-4}$ part of the delivered dose/g of kidney tissue), owing to be attributed to around 40% renal elimination of the drug (Laeis et al., 2001). Observed low concentration of olmesartan in kidneys at 8.0 h post dose of CMC suspension compared to nanoemulsion delivery may be due to shorter half-life of the drug through CMC suspension delivery. Shorter half-life of the drug in suspension group should further be explained by low distribution in several organs tested (liver, lungs and kidneys) in animals received 0.25% (*v:w*) CMC suspension of olmesartan medoxomil as compared to the nanoemulsion treated group.

Level of olmesartan in brain following oral administration of olmesartan medoxomil suspension was found to be below detectable limit at 0.5 h and 8.0 h post administration, whereas, the drug concentration at 2.0 h post administration was found to be 0.04 ± 0.007 μg/g of tissue sample ($1.1 \times 10^{-6}$ part of the delivered dose/g of liver tissue), which was significantly less ($p < 0.05$) than the nanoemulsion treated group. Fig. 3 showed the distribution pattern of olmesartan in perfused organ samples collected at different time points following single dose oral administration of nanoemulsion (10 mg/kg body weight) and CMC suspension (30 mg/kg body weight); which indicated that olmesartan underwent a rapid and wide distribution in the organs via nanoemulsion formulation. Subsequently, olmesartan permeated the rigid lipid layer of blood brain barrier (BBB) in nanoemulsion treated animals, therefore, the distribution of olmesartan in brain was significantly higher ($p < 0.05$) in nanoemulsion treated group (Table 2). This can be ascribed to the encapsulation of olmesartan medoxomil in the oily compartment of nanoemulsion that favoured its BBB permeation as well as local availability in brain (Vyas et al., 2008). Our results are in agreement with the observations by Vyas and coworkers, who reported higher brain permeability of saquinavir following its oral administration using nanoemulsion formulation approach (Vyas et al., 2008).

Presence of olmesartan in brain compartments can affect the renin angiotensin system (RAS; an important target for the AT1 receptor antagonists) that produces beneficial therapeutic effect on the pathophysiological conditions of the ischemic and memory dysfunction in hypertensive patients as evidenced by enhanced cognitive functions and improved cerebral autoregulation (Matsumoto et al., 2010; Nakagawa et al., 2015).

Clinical studies reported in this area also revealed that the angiotensin II ATI receptor blockers are potential group of medicine, which could be used to improve the memory function in hypertensive patients' independent of its blood pressure lowering action (Katada et al., 2014; Villapol et al., 2015; Davies et al., 2011). A recent report by Kono et al. on ischemic stroke rat brain model depicted that higher dose of oral lipid-soluble angiotensin receptor blocker is mandatory to successfully maintain the blood pressure of experimental animals. Authors also reported that this activity subsequently reduces the inflammation in animals after transient middle cerebral artery occlusion in stroke-resistant spontaneously hypertensive rats (Kono et al., 2015). Results from our experiments can be correlated with the fact that the nanoemulsion mediated oral delivery of olmesartan can successfully maintain the blood concentration to such a level that can successfully control the hypertension for a prolonged period of time (approximately 12.28 ± 1.320 h; Gorain et al., 2014). Subsequently, it can also be hypothesized that the presence of such a higher concentration of olmesartan in brain can significantly attenuate the activation of AT1 receptors, thereby, decreasing the central blood pressure, and in addition, it will aid in performance in learning and memory paradigms (Tota et al., 2012; Villapol et al., 2015). These prospective pharmacological activities of olmesartan medoxomil nanoemulsion opens up newer treatment opportunities for the management of brain ischemia, as well as hypertension induced memory loss.

### 3.2. Twenty-eight-day sub-chronic oral toxicity in rats

Olmesartan medoxomil is one of the well-known drug, which is widely applied to control blood pressure in hypertensive patients. Reformulation of existing oral formulation of olmesartan medoxomil using nanoemulsion approach makes it necessary to conduct the repeated dose sub-chronic toxicity study in experimental rats. The experimental observations for the toxicity assessment are discussed in subsequent sections of this manuscript.

#### 3.2.1. Cage side observation and body weight measurement

Oral administration of high dose (60 mg/kg/day), medium dose (30 mg/kg/day) and low dose (15 mg/kg/day) nanoemulsion formulation; and the administration of high dose (150 mg/kg/day), medium dose (75 mg/kg/day) and low dose (40 mg/kg/day) 0.25% CMC suspension of olmesartan medoxomil in the respective animal groups did not produce any notable changes in the open cage observations, like: animal behaviour, skin effect, lacrimation, breathing, tremors, defecation, convulsions, eyes/pupil size, palpebral closure, postural abnormalities, faeces, urine and yellowing or loss of hair and sensory-motor reflexes, like behaviour during handling, touch response, approach response, audition, pinna reflex, vision, vocalization, coordination of movements and pain perception. Notably, no mortality of animal was observed in any groups within the period of our experimentation (Table S3).

It has been established that the weight loss of experimental animals indicates toxic effects of investigational agents (Chevrier et al., 2000). Therefore, the body weight of the animals in each group was measured weekly to determine the effect on weight loss following administration of developed olmesartan medoxomil formulations. The mean body weight of olmesartan treated animals and the control animals (without treatment) was found to be increased gradually during the experimental period, however, this increase in body weight was found to be clinically insignificant, when compared with the '0' day data of respective group. The effect of olmesartan medoxomil nanoemulsion formulation on the body weight of experimental animals has been represented in Fig. 4.

Further, the chronic oral administration of olmesartan medoxomil developed the enteropathy leading to severe and chronic diarrhoea and weight loss, which were absent in case of nanoemulsion as well as CMC suspension formulations treated groups. Tran and Li reported the development of villous atrophy following prolonged exposure of olmesartan (Tran and Li, 2014). However, these unusual effect was not noted in any of the experimental

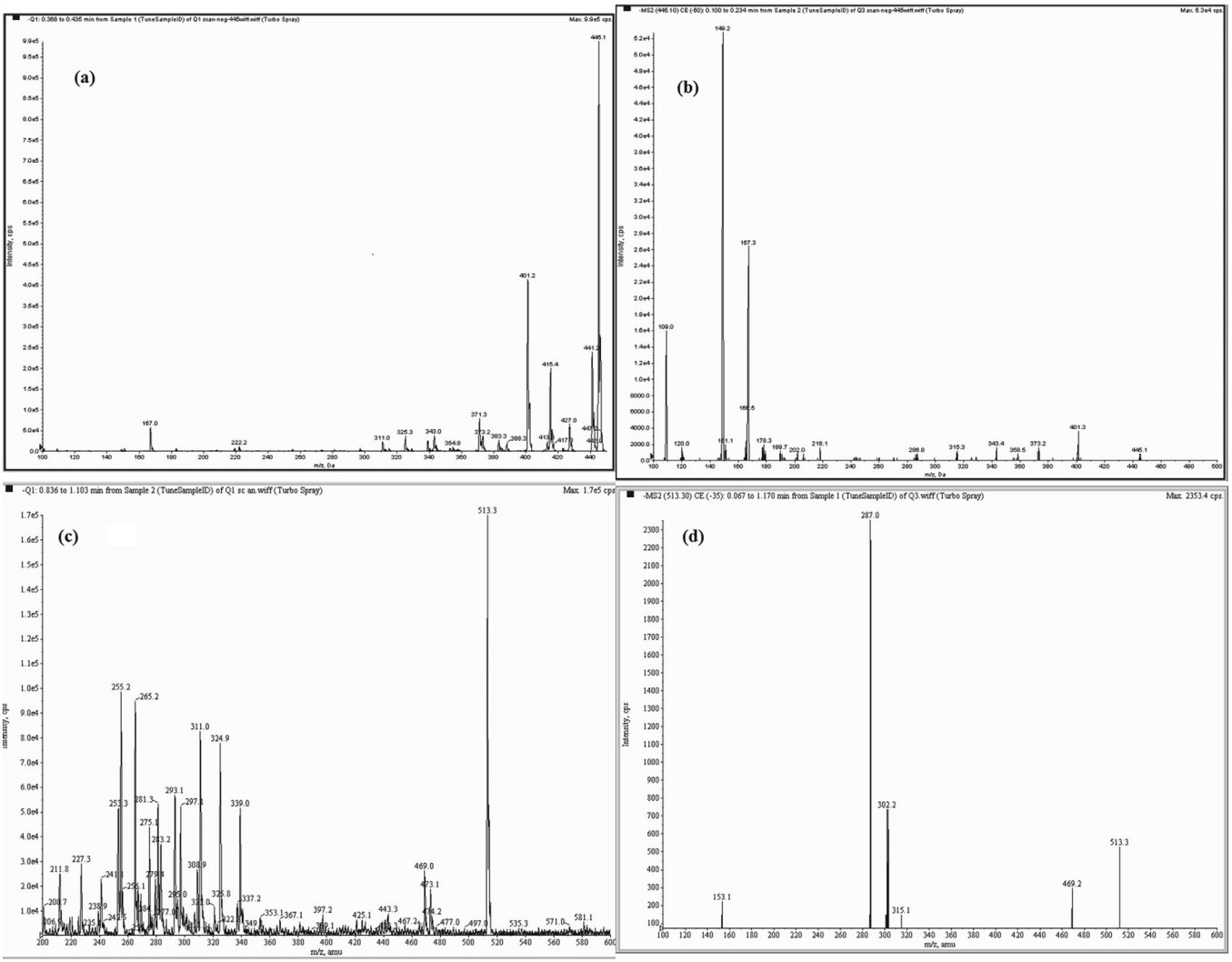


**Fig. 1.** Parent ion scanning mass spectra of (a) olmesartan; (c) telmisartan (IS); Product ion scanning mass spectra of (b) olmesartan and (d) telmisartan (IS).

animal within the time frame of our experimentation.

### 3.2.2. Food and water consumption

It has been observed that the food consumption is directly proportional to the change in body weight of the experimental animals (Morton et al., 2014). Within the time frame of our experimental condition, body weight of the animals were not affected/declined, rather a gradual increase in body weight of the animals was observed. The overall food consumption and water intake was not affected in any treatment groups as compared to the self-controls ('0' day body weight) (Table S4). The food intake findings are comparable with the findings on body weight of the animals. Moreover, it is clear that the oral administration of nanoemulsion did not affect the appetite and/or thirst of the treated animals. So, it can be concluded that the oral administration of our developed nanoemulsion formulation containing olmesartan medoxomil did not cause any notable adverse effect in the gastrointestinal tract. The entrapment of olmesartan medoxomil in the oily reservoir of nanoemulsion further prevents the direct contact of olmesartan with the intestinal villi, which is most possible reason for the de-esterification of olmesartan medoxomil (Gorain et al., 2013; Tran and Li, 2014). Hence, this nanoemulsion approach with improved bioavailability and gastrointestinal effectiveness could be a better alternative to the conventional olmesartan medoxomil formulations currently available in the market for the treatment of hypertension.

### 3.2.3. Organ-body weight index

Evaluation of organ weights is one of the essential parameter that is widely assessed to determine the toxicological findings of the chemicals and pharmaceuticals. It is well established that changes in the organ weight deciphers a direct effect of test substance on that particular organ (Sellers et al., 2007). However, this must be carefully noted that the comparison of organ weight becomes questionable when there is a manifestation of animal body weight alteration (Sellers et al., 2007).

Change in organ weights always reflected by change in body weight of the animals. Hence, the interpretation of pathological conditions based on the findings of organ weight cannot produce reproducible results. Hence, the ratio of organ weight to body weight has been widely considered to be more useful parameter by the toxicologists (Bailey et al., 2004).

For toxicological evaluation of the developed olmesartan medoxomil nanoemulsion formulation, vital organs were isolated from the experimental animals on 29th day followed by the collection of blood for the assessment of haematological and

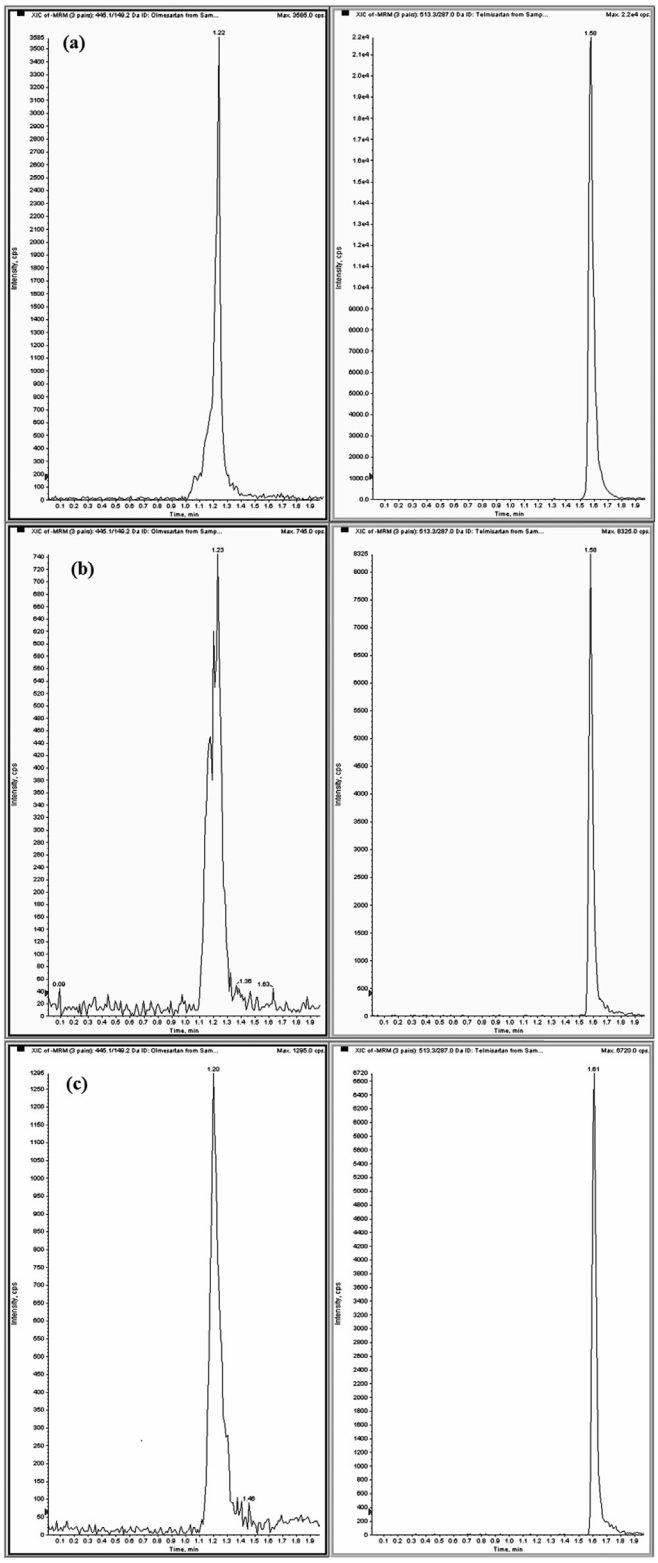


**Fig. 2.** Typical MRM chromatograms of olmesartan (left panel) and IS (right panel) in (a) cerebellum, (b) rest of the brain and (c) cortex.

biochemical parameters. Representation of organ to body weight indices for the animals received treatment of nanoemulsion and suspension are depicted in Table 3. As compared to controls, no significant changes in the relative organ weight (liver, kidney and

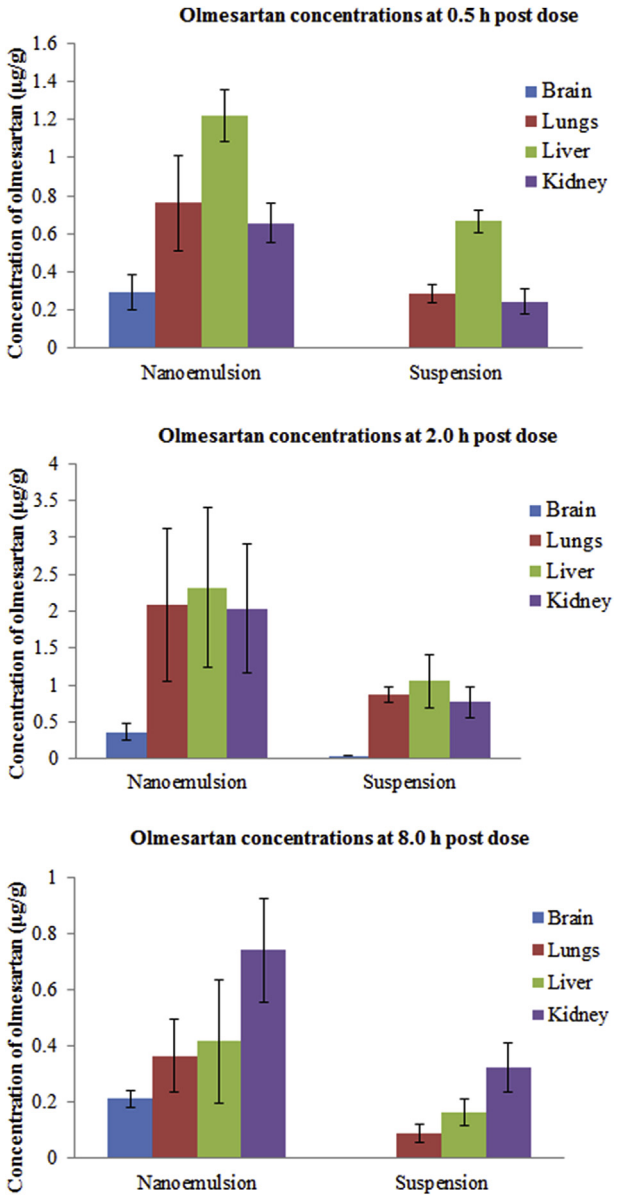


**Fig. 3.** Distribution of olmesartan in different tissues at times (i) 0.5 h; (ii) 2.0 h; and (iii) 8.0 h following oral administration of nanoemulsion and 0.25% CMC suspension of olmesartan medoxomil in rats (n = 4).

heart) to corresponding animal weight ratio was observed ($p > 0.05$). These findings suggest that the olmesartan medoxomil nanoemulsion formulation is safe to the experimental animals throughout the toxicity study period.

### 3.2.4. *Clino-pathological findings*

In addition to the organ-body weight index, pathological findings on the experimental animals help in predicting the forthcoming toxicity risks in humans. In this investigation, blood samples from the experimental animals were analysed to understand the effect of formulation of haematological and biochemical parameters of experimental animals. The findings of our subchronic oral toxicity of the formulated and optimized nanoemulsion and comparative suspension are presented in the connective sections.

**Table 2**
Concentration of olmesartan in brain after an oral administration of nanoemulsion (10 mg/kg body wt.) and suspension (30 mg/kg body wt.) of olmesartan medoxomil.

| Time | Concentration of olmesartan in brain following administration of | | |
|---|---|---|---|
| | Nanoemulsion of olmesartan medoxomil (μg/g)[a] | Suspension of olmesartan medoxomil (μg/g)[a] | *p* value[b] |
| 0.5 h | 0.290 ± 0.089 | BDL | <0.05 |
| 2.0 h | 0.333 ± 0.071 | 0.041 ± 0.007 | <0.05 |
| 8.0 h | 0.217 ± 0.062 | BDL | <0.05 |

[a] Data presented as the mean ± standard deviation (n = 4).
[b] Statistical analysis was performed by comparing two groups. BDL: below detectable limit.

*3.2.4.1. Haematological and biochemical markers.* Haematology refers to the study of the morphological and physiological characteristics of blood. Therefore, haematological parameters are one of the important measures of toxicity screening, which are useful to correlate the pathological findings with the observed physiological abnormalities in the experimental subjects. The tabular representations of all the hematological parameters obtained after sub-chronic toxicity study (28 days) following oral administration of the olmesartan medoxomil nanoemulsion and suspension are illustrated in Table 4 and Table 5. It was observed that the changes in haematological parameters remain unaffected following the treatment of high (60 mg/kg/day), medium (30 mg/kg/day) and low (15 mg/kg/day) dosage of olmesartan medoxomil nanoemulsion and administration of high (150 mg/kg/day), medium (75 mg/kg/day) and low (40 mg/kg/day) doses of 0.25% CMC suspension of olmesartan medoxomil in respective group of animals for a period of 28 days.

Liver and kidneys are vital organs that plays pivotal role in various metabolic processes of physiological system. The liver plays a vital role in xenobiotic metabolism, while kidneys are sites for filtration and re-absorption. Hence, the effect of nanoemulsion on liver and kidney was evaluated with special emphasis directed towards the function of these organs. In this context, transaminases [serum glutamate pyruvate transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT)] are considered to be widely accepted markers of liver damage (Adak and Gupta, 2009).

We have evaluated the impact of olmesartan medoxomil nanoemulsion formulation on the function of liver and kidney. Moreover, the effect of chronic oral administration of olmesartan medoxomil nanoemulsion and suspension at all dose levels was also evaluated for a period of 28 days in Wistar rats of both sex groups. The administration of olmesartan medoxomil nanoemulsion and suspension at all dose levels in respective groups of animals did not produce any notable alterations during the liver and renal function evaluations (Table 6). There were no harmful changes in the level of SGOT and SGPT were observed in the serum of the nanoemulsion as well as suspension treated groups. Hence, it is clear that the olmesartan medoxomil nanoemulsion did not affect the liver function significantly of experimental rats ($p > 0.05$). Similarly, kidney function parameters such as blood urea nitrogen, total protein and creatinine levels did not show any marked signs of

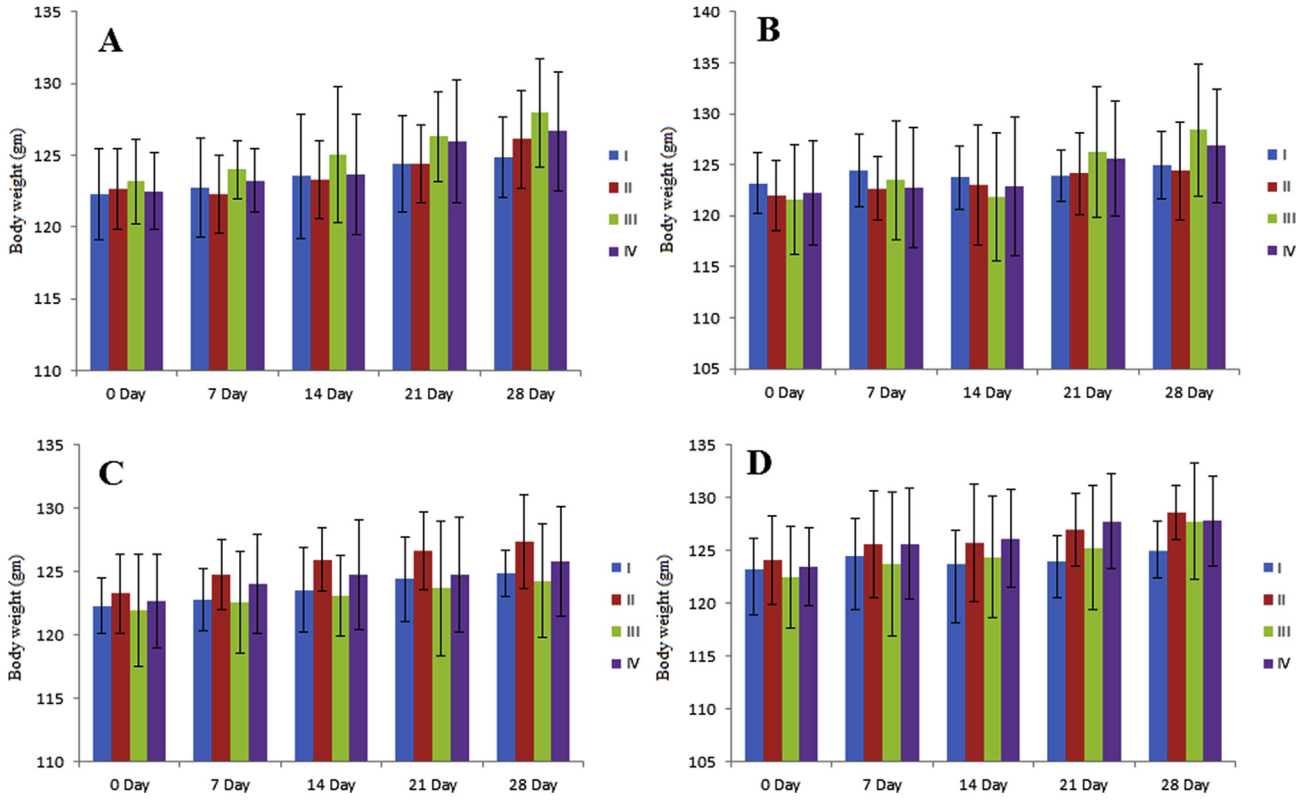


**Fig. 4.** Change in body weight of the (A) male animals treated with nanoemulsion; (B) female animals treated with nanoemulsion; (C) male animals treated with suspensions; (D) female animals treated with suspensions, where, I represents control/no treatment group; II, III and IV represent low, medium and high dose treatment of respective formulations, respectively.

**Table 3**
Organ to body weight of the animals collected after sacrificing the animals treated with olmesartan nanoemulsion and suspension, where, I represents control/no treatment group; II, III and IV represent low, medium and high dose treatment of respective formulations, respectively.

| Group No. | Sex | Nanoemulsion | | | Suspension | | |
|---|---|---|---|---|---|---|---|
| | | Liver | Kidney | Lung | Liver | Kidney | Lung |
| I | Male | 4.05 ± 0.40 | 0.61 ± 0.04 | 0.37 ± 0.03 | 4.05 ± 0.40 | 0.61 ± 0.04 | 0.37 ± 0.03 |
| | Female | 4.44 ± 0.25 | 0.59 ± 0.05 | 0.41 ± 0.03 | 4.44 ± 0.25 | 0.59 ± 0.05 | 0.41 ± 0.03 |
| II | Male | 4.40 ± 0.36 | 0.57 ± 0.05 | 0.39 ± 0.03 | 4.35 ± 0.38 | 0.57 ± 0.05 | 0.40 ± 0.03 |
| | Female | 4.35 ± 0.49 | 0.62 ± 0.03 | 0.39 ± 0.03 | 4.28 ± 0.46 | 0.61 ± 0.02 | 0.40 ± 0.02 |
| III | Male | 4.20 ± 0.34 | 0.60 ± 0.03 | 0.36 ± 0.04 | 4.30 ± 0.31 | 0.60 ± 0.03 | 0.36 ± 0.04 |
| | Female | 4.16 ± 0.45 | 0.63 ± 0.03 | 0.38 ± 0.03 | 4.18 ± 0.67 | 0.63 ± 0.03 | 0.38 ± 0.02 |
| IV | Male | 4.13 ± 0.31 | 0.60 ± 0.02 | 0.38 ± 0.02 | 4.21 ± 0.28 | 0.61 ± 0.03 | 0.39 ± 0.02 |
| | Female | 4.09 ± 0.33 | 0.62 ± 0.03 | 0.39 ± 0.02 | 4.17 ± 0.30 | 0.61 ± 0.04 | 0.41 ± 0.02 |

**Table 4**
Hematological parameters of different groups of animals after 28 days treatment with olmesartan nanoemulsion and olmesartan suspension, where, I represents control/no treatment group; II, III and IV represent low, medium and high dose of treatment regimen, respectively.

| Group No. | | Sex | Hb (g/dL) | Total RBC (x10/mm$^3$) | Rt (%) | HCT (%) | MCV μm$^3$ | MCH (pg) | MCHC (g/dL) |
|---|---|---|---|---|---|---|---|---|---|
| Control | I | Male | 14.38 ± 1.22 | 6.20 ± 0.66 | 2.43 ± 0.43 | 43.63 ± 4.14 | 53.37 ± 2.53 | 19.10 ± 1.28 | 34.75 ± 1.39 |
| | | Female | 14.68 ± 1.88 | 6.23 ± 0.70 | 2.20 ± 0.50 | 44.15 ± 5.30 | 53.78 ± 3.44 | 18.95 ± 1.08 | 33.90 ± 1.24 |
| Nanoemulsion | II | Male | 14.20 ± 1.44 | 6.05 ± 1.06 | 1.83 ± 0.42 | 43.45 ± 4.84 | 53.65 ± 3.68 | 19.58 ± 1.41 | 34.48 ± 1.59 |
| | | Female | 14.63 ± 1.50 | 5.97 ± 0.89 | 2.43 ± 0.47 | 43.78 ± 4.77 | 52.80 ± 3.16 | 19.18 ± 1.42 | 33.70 ± 2.22 |
| | III | Male | 15.23 ± 0.66 | 5.92 ± 0.40 | 2.23 ± 0.41 | 45.18 ± 2.12 | 53.93 ± 3.05 | 19.25 ± 1.09 | 34.20 ± 1.02 |
| | | Female | 15.53 ± 0.87 | 6.03 ± 0.74 | 2.32 ± 0.37 | 46.58 ± 2.63 | 55.77 ± 2.41 | 19.48 ± 1.22 | 34.53 ± 1.57 |
| | IV | Male | 14.53 ± 1.70 | 6.38 ± 1.51 | 2.45 ± 0.48 | 43.55 ± 5.58 | 52.97 ± 2.40 | 18.43 ± 1.84 | 33.80 ± 1.56 |
| | | Female | 14.43 ± 1.31 | 7.05 ± 1.00 | 2.18 ± 0.42 | 43.70 ± 3.69 | 53.87 ± 3.71 | 18.72 ± 2.56 | 34.98 ± 1.74 |
| Suspension | II | Male | 14.92 ± 2.27 | 6.28 ± 1.53 | 2.33 ± 0.47 | 44.72 ± 7.29 | 53.47 ± 3.55 | 18.98 ± 1.32 | 34.50 ± 1.09 |
| | | Female | 14.02 ± 1.50 | 7.43 ± 0.87* | 2.05 ± 0.38 | 41.53 ± 4.67 | 53.03 ± 3.73 | 18.83 ± 2.56 | 34.82 ± 1.59 |
| | III | Male | 14.35 ± 1.76 | 5.68 ± 1.36 | 1.90 ± 0.55 | 43.40 ± 4.49 | 54.60 ± 2.67 | 19.30 ± 1.36 | 35.02 ± 1.58 |
| | | Female | 14.93 ± 1.30 | 7.07 ± 0.95 | 2.27 ± 0.45 | 44.47 ± 4.07 | 54.63 ± 3.55 | 19.08 ± 2.57 | 35.00 ± 1.27 |
| | IV | Male | 13.80 ± 1.82 | 5.48 ± 0.62 | 1.68 ± 0.25* | 41.82 ± 4.86 | 54.58 ± 2.84 | 19.23 ± 1.27 | 34.88 ± 1.92 |
| | | Female | 14.67 ± 1.35 | 6.73 ± 0.82 | 2.42 ± 0.52 | 45.08 ± 4.32 | 53.93 ± 3.77 | 19.07 ± 1.90 | 34.62 ± 0.76 |

Hb: Hemoglobin, RBC: Red blood corpuscles, Rt: Reticulocyte, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration. The data presented in this table as mean ± S.D., n = 6. * signifies $p<0.05$.

alteration. Thus, it can be concluded from these findings that the developed olmesartan medoxomil nanoemulsion is nontoxic to the experimental animals within the time frame of 28 days of sub-chronic toxicity study.

*3.2.4.2. Histopathology.* Histopathology is performed to study the microscopic features of tissues for any abnormalities into the organs (Greaves, 2011). In this study, we have investigated the impact of repeated oral administration of olmesartan medoxomil nanoemulsion on the experimental animals. The histopathological analysis of liver showed that hepatocytes are arranged in cords around hepatic venules to form hepatic lobules. The portal tract and sinusoidal space are within normal limit. No evidence of any pathological lesions were seen in the histocultures of the experimental animals. Besides, kidney sections of the animals showed no alteration except the presence of normal glomeruli, renal tubules, interstitial tissue and blood vessels along with renal pyramid in all the test groups.

Further, sections of stomach showed the histology of normal stomach and oesophagus comprising of mucosal layer, sub mucosa, muscular propria and serosa. No evidence of any ulcerating or malignant process was observed at the end of the study period.

**Table 5**
Hematological parameters of different groups of animals after 28 days treatment with olmesartan nanoemulsion and olmesartan suspension, where, I represents control/no treatment group; II, III and IV represent low, medium and high dose of treatment regimen, respectively (continued hematological parameters).

| Group No. | | Sex | Platelets (10$^3$/μL) | Total WBC (10$^3$/μL) | Differential count (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | N | L | E | M |
| Control | I | Male | 7.33 ± 0.93 | 6.87 ± 1.26 | 21.83 ± 3.66 | 75.50 ± 3.39 | 1.33 ± 0.52 | 1.33 ± 0.52 |
| | | Female | 7.75 ± 1.03 | 6.30 ± 0.94 | 20.83 ± 6.01 | 76.50 ± 5.47 | 1.83 ± 1.33 | 0.83 ± 0.75 |
| Nanoemulsion | II | Male | 7.63 ± 1.03 | 6.88 ± 0.84 | 19.17 ± 3.19 | 78.17 ± 3.54 | 1.33 ± 0.52 | 1.33 ± 0.52 |
| | | Female | 7.17 ± 0.84 | 6.78 ± 1.72 | 22.83 ± 3.97 | 74.50 ± 3.62 | 1.33 ± 0.52 | 1.33 ± 0.52 |
| | III | Male | 6.95 ± 0.97 | 5.72 ± 1.51 | 24.17 ± 2.71 | 72.83 ± 2.93 | 2.17 ± 0.75 | 0.83 ± 0.75 |
| | | Female | 6.83 ± 0.58 | 6.53 ± 1.59 | 17.33 ± 4.08 | 79.50 ± 4.89 | 2.50 ± 1.05 | 0.67 ± 0.52 |
| | IV | Male | 6.63 ± 1.06 | 6.13 ± 1.57 | 23.17 ± 4.71 | 73.83 ± 4.22 | 1.83 ± 0.75 | 1.17 ± 0.98 |
| | | Female | 7.08 ± 0.81 | 6.35 ± 1.21 | 22.50 ± 3.62 | 74.83 ± 2.79 | 1.83 ± 0.98 | 0.83 ± 0.75 |
| Suspension | II | Male | 6.92 ± 1.24 | 5.83 ± 1.89 | 22.33 ± 3.56 | 74.67 ± 2.80 | 1.83 ± 0.75 | 1.17 ± 0.98 |
| | | Female | 6.75 ± 0.61 | 6.23 ± 1.48 | 21.33 ± 2.50 | 75.33 ± 2.94 | 2.50 ± 1.38 | 0.83 ± 0.75 |
| | III | Male | 7.65 ± 1.28 | 6.48 ± 0.64 | 19.83 ± 3.54 | 77.33 ± 4.23 | 1.67 ± 0.52 | 1.17 ± 0.75 |
| | | Female | 6.85 ± 0.59 | 6.37 ± 1.27 | 19.00 ± 3.46 | 77.83 ± 4.54 | 2.50 ± 1.38 | 0.67 ± 0.52 |
| | IV | Male | 8.10 ± 0.94 | 6.40 ± 0.57 | 19.17 ± 3.43 | 77.67 ± 4.08 | 1.83 ± 1.17 | 1.33 ± 0.52 |
| | | Female | 6.97 ± 0.49 | 6.95 ± 1.38 | 20.83 ± 3.31 | 76.67 ± 2.58 | 1.50 ± 0.55 | 100 ± 0.63 |

WBC: White blood corpuscles, N: Neutrophils, L: Lymphocytes, E: Eosinophils, M: Monocytes. The data presented in this table as mean ± S.D., n = 6.

**Table 6**
Biochemistry parameters of different groups of animals after 28 days treatment with olmesartan nanoemulsion and olmesartan suspension, where, I represents control/no treatment group; II, III and IV represent low, medium and high dose of treatment regimen, respectively.

| Group No. | | Sex | Total Serum Protein (g/dL) | BUN (mg/dL) | SGPT (IU/L) | SGOT (IU/L) | Creatinine (mg/dL) | Total Bilirubin (mg/dL) |
|---|---|---|---|---|---|---|---|---|
| Control | I | Male | 6.48 ± 0.57 | 17.83 ± 2.79 | 50.67 ± 7.58 | 98.33 ± 10.63 | 0.72 ± 0.21 | 0.38 ± 0.15 |
| | | Female | 6.22 ± 0.72 | 16.17 ± 3.87 | 50.83 ± 7.22 | 95.33 ± 16.16 | 0.68 ± 0.19 | 0.38 ± 0.12 |
| Nanoemulsion | II | Male | 6.45 ± 0.77 | 16.17 ± 4.02 | 48.17 ± 8.75 | 94.67 ± 14.53 | 0.67 ± 0.16 | 0.42 ± 0.10 |
| | | Female | 6.57 ± 0.48 | 16.50 ± 3.94 | 50.50 ± 7.56 | 91.33 ± 13.26 | 0.77 ± 0.21 | 0.35 ± 0.14 |
| | III | Male | 6.30 ± 0.68 | 16.17 ± 2.48 | 48.67 ± 4.76 | 94.50 ± 14.38 | 0.77 ± 0.18 | 0.37 ± 0.12 |
| | | Female | 6.12 ± 0.53 | 18.17 ± 3.06 | 51.17 ± 7.22 | 93.33 ± 10.41 | 0.72 ± 0.23 | 0.38 ± 0.08 |
| | IV | Male | 6.17 ± 0.58 | 17.17 ± 3.76 | 52.00 ± 7.29 | 90.17 ± 15.34 | 0.75 ± 0.19 | 0.40 ± 0.14 |
| | | Female | 6.20 ± 0.65 | 18.50 ± 2.07 | 48.00 ± 9.32 | 93.67 ± 10.25 | 0.65 ± 0.19 | 0.35 ± 0.14 |
| Suspension | II | Male | 5.92 ± 0.69 | 16.00 ± 3.22 | 47.33 ± 5.35 | 85.83 ± 13.93 | 0.73 ± 0.21 | 0.37 ± 0.12 |
| | | Female | 6.50 ± 0.73 | 19.33 ± 2.66 | 53.00 ± 12.10 | 89.83 ± 11.86 | 0.65 ± 0.19 | 0.43 ± 0.08 |
| | III | Male | 6.37 ± 0.65 | 17.67 ± 3.08 | 50.67 ± 9.18 | 93.83 ± 13.48 | 0.73 ± 0.19 | 0.32 ± 0.16 |
| | | Female | 6.40 ± 0.78 | 17.33 ± 2.94 | 48.83 ± 7.94 | 92.67 ± 12.16 | 0.73 ± 0.21 | 0.33 ± 0.12 |
| | IV | Male | 6.18 ± 0.83 | 16.33 ± 3.67 | 46.50 ± 7.84 | 87.17 ± 9.00 | 0.68 ± 0.22 | 0.33 ± 0.15 |
| | | Female | 6.10 ± 0.84 | 17.67 ± 2.16 | 47.83 ± 5.00 | 89.83 ± 11.16 | 0.75 ± 0.16 | 0.38 ± 0.13 |

BUN: Blood urea nitrogen, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase. The data presented in this table as mean ± S.D., n = 6.

Lungs sections showed histology of normal lung tissue comprising of different division of respiratory trees up to alveolar duct, alveoli, interstitial tissue and blood vessels. No evidence of any granuloma or neoplastic process was seen in the lung histoculture. Representative pictures of histopathological examination of liver, kidney, stomach and lung in high dose nanoemulsion and suspension

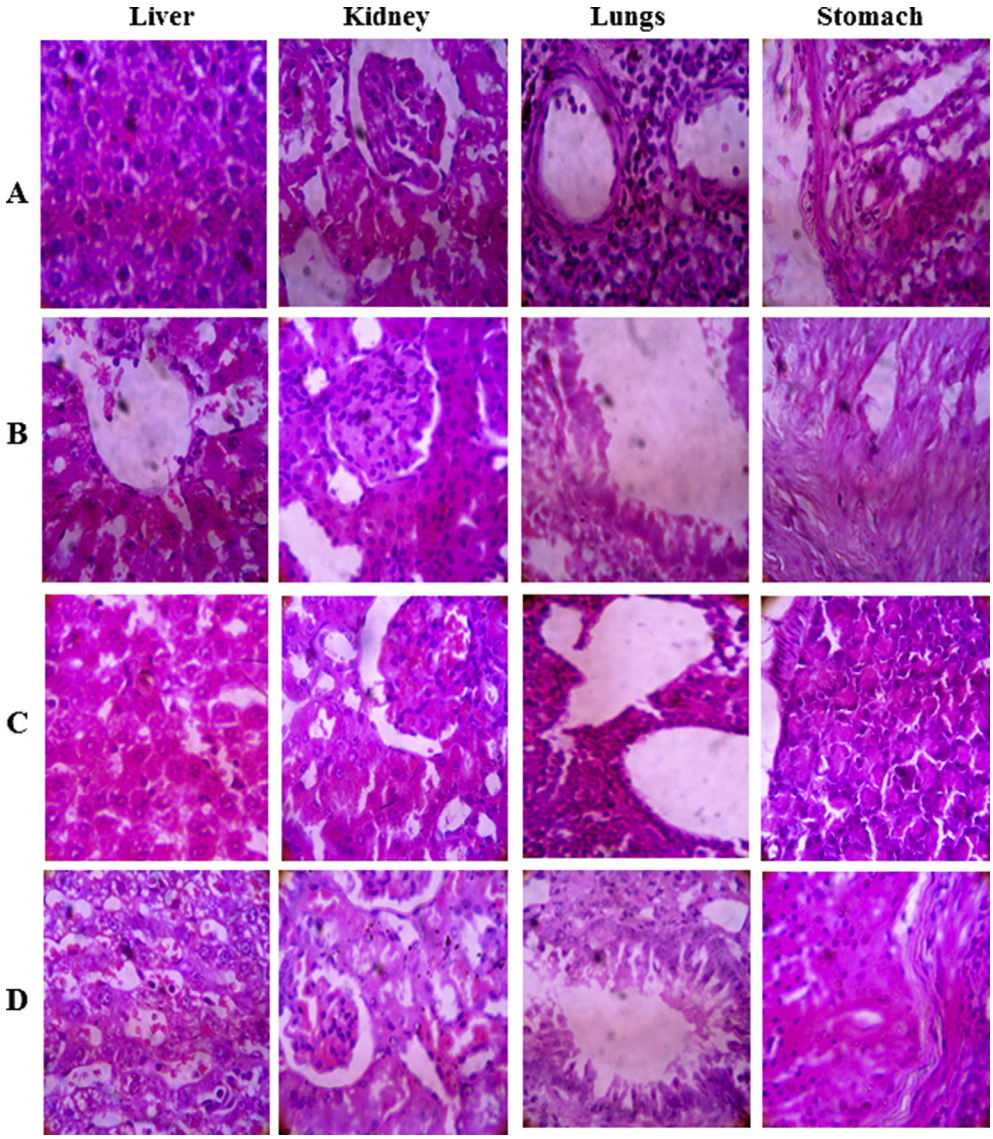


**Fig. 5.** Histopathological section of liver, kidney, lungs and stomach showing normal histological picture of animals treated with (A) high dose oral olmesartan nanoemulsion in female rats, (B) high dose oral olmesartan nanoemulsion in male rats, (C) high dose oral olmesartan suspension in female rats, (D) high dose oral olmesartan suspension in male rat.

treated male and female animals are shown in Fig. 5.

The outcomes of histopathological cross sections revealed that the oral administration of olmesartan medoxomil nanoemulsion formulation did not show any evidence of pathological lesion in any of the tissue section of liver, kidney, stomach and lung of any groups of experimental animals. Hence, it could be summarised from the findings that the nanoemulsion formulation may be considered as relatively safe of toxicity, as it did not cause any lethality nor produced any remarkable haematological, biochemical and structural adverse effects in the experimental Wistar rats within the entire period of 28-days sub-chronic toxicity study.

## 4. Conclusion

In our previous manuscript, we reported the preparation of an *o/w* nanoemulsion of olmesartan medoxomil which elicited the aptitude of solubility enhancement, improved oral bioavailability and resolution of unfavorable stability issues of olmesartan medoxomil in the gastric juice to its less permeable olmesartan base. Our solubility augmentation technique coupled with permeability enhancement strategy improved the systemic exposure of olmesartan through oral route that simultaneously increase the concentration of drug in different perfused tissues, including brain. The clinical study, haematological study, organ to body mass indices, macroscopical evaluation and histopathology data obtained from 28 day sub-chronic toxicity study established that the formulation is safe and did not elicit any noticeable toxicity. It is recommended that the developed formulation bears high merits to be further explored for its clinical investigation on higher animal models with an anticipation to replace the available solid (tablet) formulation of olmesartan medoxomil for the treatment of hypertension in more economical and effective manner. Additional research can be designed to focus completely towards understanding the mechanism of brain penetration and systematic engagement of this nanoformulation approach in the cerebral ischemia and memory dysfunction in hypertensive patients, to improve the quality of life of those patients.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2016.10.020.

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2016.10.020.

## Conflict of interest

The authors confirm that this article content has no conflicts of interest.

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