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## RESEARCH ARTICLE

### Single Time Heated Different Vegetable Oils Use-Impact on the Magnitude of Total Parenteral Nutrition (TPN) Associated Adverse Effects

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#### Abstract:

#### Background:

In clinical practice, Total Parenteral Nutrition (TPN) is standard of care for patients of gastrointestinal tract disorders. TPN therapy is associated with a number of adverse effects like hepatobiliary dysfunction, dyslipidemia, and oxidative stress. Different vegetable oils are used by every individual daily in routine life.

#### Objective:

This study assessed the magnitude of TPN-associated adverse effects in animals fed on different vegetable oils.

#### Methods:

Healthy adult rabbits of local strain were divided into 5 groups (n= 8). The study was divided into 2 phases. Phase I: oil feeding; the first set of rabbits served as control and fed on a normal rabbit diet. Four sets of rabbits were treated for 16 weeks with 1 ml/kg/day of single time heated olive (STH-OO), canola (STH-CO), sunflower oils (STH-SO) or a mixture of these oils (STH-MVO). In phase II: TPN was given to each group, including the control group, for 2 weeks. Before and after TPN therapy, body and liver weights were measured. Plasma lipid profile [triglycerides, total cholesterol, high-density lipoproteins, low-density lipoproteins, very-low-density lipoproteins], liver function marker [total-protein, albumin, total and direct bilirubin, serum glutamic pyruvic transaminase, serum glutamic-oxaloacetic transaminase, gamma-glutamyl transferase, and alkaline phosphatase], oxidative stress and tissue damage parameters [malondialdehyde, C-reactive protein, lactate dehydrogenase, and creatine phosphokinase] of all the groups were measured at the end of TPN therapy.

#### Results:

Significantly ( $p < 0.05$ ) elevated hepatic enzymes, oxidative stress and tissue damage markers along with dyslipidemia were observed in STH-MVO and STH-SO fed groups, in comparison to control. In STH-OO and STH-CO groups, all these parameters were insignificantly different than control after 2 weeks of TPN therapy. The higher plasma levels of, High-Density Lipoproteins (HDL), total protein and albumin and reduced Malondialdehyde (MDA) levels, were observed in STH-OO and STH-CO groups than the control.

#### Conclusion:

This study recommends that high monounsaturated fatty acids containing oils like olive and canola oils, are found to have strong resistance against the hepatic injury and lipid peroxidation. The study also recommends taking the history of oil use by an individual before the initiation of therapeutic agents with known side effects of hepatic toxicity and dyslipidemia.

**Keywords:** Total Parenteral Nutrition (TPN), Hepatobiliary disorders, Poly Unsaturated Fatty Acids (PUFA), Monounsaturated Fatty Acids (MUFA), High-Density Lipoproteins (HDL), Malondialdehyde (MDA) levels.

#### Article History

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## 1. BACKGROUND

Total Parenteral Nutrition (TPN) is a lifesaving modality by preventing and curing the nutritional deficiencies in individuals with gastrointestinal tract disorders [1]. Hepato-

biliary dysfunction, cirrhosis, and hepatic failure are well-known TPN associated complications. A study has reported TPN-induced classical picture of cholestasis, fibrosis, and portal inflammation [2]. These hepatobiliary changes are mostly multifactorial and mainly due to the exclusion of enteral

nutrition, infections, excessive biliary secretions, TPN composition, and toxic TPN bag materials like plasticizers of Polyvinyl Chloride (PVC) [3, 4]. In addition, the nature of lipid emulsion, as the dense energy source in TPN, may also contribute to these complications [5]. In Pakistan, commercially available Intravenous Lipid Emulsion (IVLE), is manufactured from soybean-based oils, which contains a higher percentage (about 62%) of Polyunsaturated Fatty Acids (PUFA) and gamma-tocopherol. High PUFA content increases oxidative stress through the free radical formation and increased production of the proinflammatory cytokines [interleukin- $\alpha$  and interleukin- $\beta$ , interleukin-6, interleukin-8, Tumor Necrosis Factor (TNF)- $\alpha$ ], and further inflammatory mediator like platelets activating factor, mostly through the metabolism of arachidonic acid [6]. These proinflammatory cytokines, especially TNF directly involves in the pathogenesis of TPN-associated cholestasis [7].

The internal chemical balance of our body is controlled by the liver [8], it detoxifies and eliminates all the chemicals including drugs and other xenobiotics [9], through conjugating, reducing or oxidizing them [10]. Free radical generation may lead to liver damage and cause hepatitis, cirrhosis, and liver cancer [11], which are amongst the leading causes of morbidity and mortality worldwide and have diverted the researchers to find the solution from nature [12]. Exposure of different environmental toxins and xenobiotics, such as acetaminophen, thioacetamide, Carbon Tetrachloride (CCl<sub>4</sub>), and alcohol harm the liver by generating Reactive Oxygen Species (ROS) [12]. Liver damage is also produced by deadly chemicals, drugs, and viral infections [13].

Several plants are used as therapeutic agents, especially in the south Asian region [14]. Studies have reported the health benefits of different edible oils like, *Moringa oleifera Lam* seed oil is reported to have hepatoprotective properties by producing antioxidants, which have scavenging potential for free radical [15]. A recently conducted animal studies reported hypocholesterolemic and hepatoprotective effects of virgin avocado oil [16], and hepatoprotective effects of coconut oil [17], antioxidant properties with better lipid metabolism of olive oil [18], along with antineoplastic properties against various cancers, like breast, stomach, ovary, colon, and endometrium [19]. Another olive oil study (used with camel milk), reported its protective effects on hepatocytes in animals with acetaminophen-induced hepatic damage [20].

We hypothesized that the type of oil used by an individual in daily life may affect the magnitude of the outcome and the adverse effects of any therapeutic agent. The present study designed to evaluate the impact of single time heated commonly used vegetable oils (either individual or in the blends) on TPN associated lipid peroxidation, oxidative stress, and hepatobiliary complications. To our information, no such effort has been reported from Karachi, Pakistan. In Pakistan, oils are mostly used with processed food, so all oils were processed through single time heating for the same duration as routine cooking time, to mimic the real-time scenario.

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## 2. MATERIALS AND METHODS

### 2.1. Phase I: Oil Feeding Model

#### 2.1.1. Oil Samples Preparation

Standard food-grade canola, sunflower, and olive oils (7 liters of each oil) were purchased from Karachi local market. Sufficient quantity of oil (4 liters from each sample) was separated and thermally treated above its smoke point [olive oil 180 °C; canola oil 200 °C; and sunflower oil 225 °C] for 45 minutes with potatoes frying and then cooled to room temperature, labeled as Single Time Heated Canola Oil (STH-CO), Single Time Heated Sunflower Oil (STH-SO) and single time heated olive oil (STH-OO) [21, 22]. Then one liter from each a single time heated oil was taken and mixed and labeled as Single Time Heated Mix Vegetable Oils (STH-MVO). To prevent photodegradation, all samples were stored in amber color bottles.

#### 2.1.2. Experimental Animals

Healthy male rabbits weighing 1450  $\pm$  10g were purchased from the department of pharmacology, University of Karachi, Pakistan. Rabbits were kept individually in wire-topped steel cages with wooden bottoms, in control conditions (temperature 23  $\pm$  2 °C and 50-60% relative humidity) for 16 weeks with an estimated 12/12 hours light/dark photo-cycle. Animals were acclimatized for seven days before starting the experiment. The animals were handled throughout according to the institutional animal ethical committee guidelines.

#### 2.1.3. Animals Grouping and Oil Feeding

The study was performed by randomly allocating rabbits into five groups (n = 8). Unexposed control rabbits were fed on fresh hay and water only (normal diet). In 4 treatment groups, animals were treated with 1 ml/kg/day of either of single time heated oils (STH-OO, STH-CO, STH-SO or STH-MVO) once daily at 10.00 am for 16 weeks (equivalent to 12 years of human life). For the rest of the day, animals of all the groups were fed ad libitum on a regular normal diet. Daily oil dose was calculated based on previous animal studies [23].

### 2.2. Phase II: Establishing a TPN Model

At the end of the oil therapy, animals' bodyweight ranged from 1630g–1680g. The rabbits were kept in an individual clean stainless-steel cage (used 70% isopropyl alcohol for the cleaning). Animals were given anesthesia by administering ketamine (dose = 60 mg/kg) intramuscularly [24]. Ear vein catheterization was performed [25] for the administration of cyclic TPN to all groups of animals, including the unexposed control group.

#### 2.2.1. TPN Solution Composition and Preparation

Certified nutrition support pharmacists performed all TPN calculations according to adult requirements. The average weight of rabbits [1650 g (range, 1630-1680 g)] was taken for TPN formula calculation. Each rabbit received 30 Kcal/kg/day, 65% from dextrose, and 35% from lipid source as non-protein calories. This provided non-protein calorie to grams of nitrogen

ratio of 160:1 [26, 27]. Doses of all the component were calculated as per standard guidelines and animal weight; amino acids were given with 0.8 g/kg/day, IVLE with 1 g/kg/day and dextrose was given as the source of glucose with Glucose Infusion Rate (GIR) of 5.5 mg/kg/minute. Total fluid volume was calculated with 50 ml/kg/day [28, 29].

TPN formulas were prepared in the piggy bags. It was a 3 in 1 formula, providing all the macro and micronutrients in one bag (Table 1) with the stability of 24 hours at room temperature. All the components were mixed under aseptic conditions daily to supply 24 hours requirement only. Each bag was labeled after preparation and wrapped with aluminum foil to protect from light [30, 31].

**Table 1. Composition of TPN bag.**

Components	Quantity / 80ml	Yield
<b>Macronutrients</b>		
10% Amino acids solution	13.5 ml	6 Kcal
25% Dextrose	20 ml	29 Kcal
10% Dextrose	34.5 ml	
20% Lipid	8.2 5 ml	15 Kcal
<b>Electrolytes and Trace Elements</b>		
14.6% Sodium chloride	1 ml	2.5 mEq = 57.5 mg
Potassium Phosphate **	0.35 ml	Potassium 1.54 mEq = 60.1 mg
		Phosphorus 1.1 Mmol = 34.1 mg
10% calcium gluconate	1.25 ml	125 mg
Magnesium Sulfate	0.1 ml	0.4 mEq = 50 mg
Zinc sulfate	0.3 ml	0.3 mg
<b>Other components</b>		
Multivitamin *	1 ml	-
Heparin	0.01 ml	50units
Total calories/80ml	-	50 Kcal

\* Multibionta was used, for all water- and fat-soluble vitamins; \*\* 1 ml Potassium phosphate contains 3Mmol Phosphate and 4.4 mEq Potassium.

### 2.2.2. TPN Administration

After 16 hours of the anesthesia recovery period, TPN infusion was started by injecting micropump, to maintain the uniform infusion rate over 12 hours from 8 am till 8 pm daily. 1ml of Normal Saline (NS) was used to flush the line before and after completion of the TPN infusion daily. IV set was replaced every 24 hours. All the syringes and tubing used for TPN administration were polyvinyl chloride-free and were protected from light. On the first day of TPN, each rabbit was given half of the volume, with an infusion rate of 4 ml/Kg/hour, with no other change in TPN composition and calculations, followed by 6 ml/kg/hour on the second day. The target rate of 8 ml/kg/hour was given from third till the fourteenth day of TPN therapy. TPN was the only provided caloric nutritional source for the study animals [30, 31].

### 2.3. Gross Monitoring

Morbidity and mortality were monitored through the study

period. Body weight was recorded at baseline and followed by weekly monitoring at weeks 4, 8, 12, 16, and 18. At the end of phase I and II three rabbits were sacrificed with the administration of intravenous sodium pentobarbital 100mg/kg followed by decapitation as per American Veterinary Medical Association (AVMA) guidelines for the euthanasia of animals [32]. Furthermore, animals were necropsied to retrieve the liver for organ weight evaluation.

### 2.3.1. Blood Sample Collection for Lipid Profile and Liver Function Markers Analysis

Blood was collected from ear veins in a fasting state at the end of the 18<sup>th</sup> week. To evaluate the hematological parameters, 2 ml blood was taken in EDTA.K3 tubes and 5 ml blood in a gel tube, then centrifugation was performed at 3000 rpm for 15min, to isolate the serum. With the use of clean, dry disposable plastic syringes, the supernatant was parted and stored at -18°C and then used to analyze the lipid profile [(Triglycerides (TG), Total Cholesterol (TC), High-Density Lipoproteins (HDL), Low-Density Lipoproteins (LDL), Very-Low-Density Lipoproteins (VLDL)] and liver function marker [total-protein, Albumin (ALB), Total Bilirubin (TB), Direct Bilirubin (DB), Serum Glutamic-Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Transaminase (SGOT), Gamma-Glutamyl Transferase (GGT), and Alkaline Phosphatase (ALP)] by using analytical kits of RANDOX Laboratories Ltd. following the manufacturer's instructions.

### 2.3.2. Oxidative Stress and Tissue Damage Parameters

Lipid peroxidation was measured by determining plasma Malondialdehyde (MDA), following Kikugawa *et al.* method [33]. The plasma C-Reactive Protein (CRP) was measured by using the ELISA kit (Abnova, Taipei, Taiwan), as per the manufacturer's instruction for use. A commercially available kit was used to analyze Lactate Dehydrogenase (LDH) levels (BioVision, USA). The samples, as well as standards, were prepared as per the manufacturer's instructions, following Hamsi *et al.* method [34]. To measure the fasting plasma homocysteine concentrations, we adopted the method described by Sheu *et al.* [35]. Creatine Phosphokinase (CPK) was measured by a commercially available kit (Beckman Coulter, Brea, USA), followed by manufacturer instructions.

### 2.4. Statistical Analysis

In our study, the final data is presented in the form of mean  $\pm$  Standard Deviation (SD). We applied Analysis of Variance (ANOVA) followed by post hoc Tukey's Honest Significant Difference (HSD) test to find out the statistical significance. A paired t-test was applied for pre and post-analysis. The value  $p < 0.05$  was considered as significant and  $p < 0.005$  as highly significant. The data analysis of this study was performed using SPSS (Statistical Product and Service Solutions) software.

## 3. RESULTS

### 3.1. Gross Observation of Animals

No death and unexpected signs and symptoms were noticed in experimental animals throughout the study period.

Increment in the body and liver weights were observed in all the groups after 2 weeks of TPN administration, but statistically significant ( $p < 0.05$ ) difference was observed in STH-SO fed animals in comparison with control and other treatment groups. On the other hand, an insignificant difference was observed in STH-OO, STH-CO, and STH-MVO groups. Comparison of body and liver weights between before and after TPN administration shows, statistically significant ( $p < 0.05$ ) weight gain in STH-SO and STH-MVO groups animals, however, in STH-OO and STH-CO groups the weight gain is statistically not significant across the TPN therapy (Table 2).

### 3.2. Biochemical Markers of Hepatic-Function

The serum levels of GGT, SGOT, SGPT, ALP, TB, and DB were elevated in all oil-fed groups after TPN administration in comparison with control. Statistically highly significant results were found in STH-SO ( $p < 0.005$ ) and STH-MVO ( $p < 0.05$ ) treated group when compared to control, STH-CO and STH-OO fed groups (Table 3).

### 3.3. Plasma Lipid Profile

Significantly ( $p < 0.005$ ) increased levels of TGs, TC, LDL-Cholesterol and VLDL-cholesterol, whereas lower HDL-cholesterol, serum protein, and Albumin (ALB) levels were observed in STH-SO and STH-MVO treated group in comparison with control and other oil-fed groups. Least variations in plasma lipid profiles were observed in STH-OO, and STH-CO fed groups (Table 3).

### 3.4. Oxidative Stress and Tissue Damage Parameters

After 14 days of TPN administration, the plasma of levels of MDA (lipid peroxidation marker), CPK, LDH and homocysteine (tissue damage markers) and CRP (inflammatory marker) were significantly elevated in STH-MVO ( $p < 0.05$ ) and STH-SO ( $p < 0.005$ ) fed groups. In these variables, no significant change was observed in STH-OO and STH-CO fed animals, as compared to control. Among all groups, tissue damage markers were found with the least variation in STH-OO fed animals in pre and post TPN administration phases (Table 3).

**Table 2. Body and liver Weights before and after 2 weeks of TPN administration (n=8).**

Groups	Before TPN	After TPN	p- values
<b>Bodyweight (g) *</b>			
Control	1631.58 (10.01)	1666.33 (8.05)	ns
STH-OO	1651.58 (10.21)	1682.56 (9.02)	ns
STH-CO	1660.89 (9.50)	1689.44 (10.02)	ns
STH-MVO	1676.02 (9.58)	1712.98 (12.53)	< 0.05
STH-SO	1680.58 (9.89)	1739.65 (8.59)	< 0.05
<b>Liver Weight (g) *</b>			
Control	55.29 (5.11)	58.21 (4.31)	ns
STH-OO	59.73 (6.35)	61.85 (3.45)	ns
STH-CO	62.08 (5.05)	63.03 (4.89)	ns
STH-MVO	68.77 (4.87)	73.03 (6.22)	ns
STH-SO	70.98 (4.68)	78.99 (6.25)	< 0.05

\*Data representation (mean  $\pm$  S.D); ns= not significant

**Table 3. Comparison of liver function markers, lipid profile, and tissue damage parameters among all the group after 2 weeks of TPN administration (n=8).**

Parameter	Control	STH-OO	STH-CO	STH-MVO	STH-SO
<b>Liver function markers</b>					
SGPT (IU/L)	88.8( $\pm$ 7.4)	89.6( $\pm$ 6.4)	102.9( $\pm$ 6.1)	195.6( $\pm$ 39.2) *	245.7( $\pm$ 4.1) **
SGOT(IU/L)	86.2( $\pm$ 8.3)	87.5( $\pm$ 3.5)	99.6( $\pm$ 5.6)	194.8( $\pm$ 54.6) *	255.8( $\pm$ 7.2) **
ALP(IU/L)	98.2( $\pm$ 5.6)	98.8( $\pm$ 8.0)	98.2( $\pm$ 5.6)	141.4( $\pm$ 6.7) *	227.8( $\pm$ 3.4) **
GGT(IU/L)	17.2( $\pm$ 2.3)	19.8( $\pm$ 1.3)	21.3( $\pm$ 3.3)	65.9( $\pm$ 12.9) **	88.1( $\pm$ 1.8) **
TB (mg/dl)	3.7( $\pm$ 0.06)	3.7( $\pm$ 0.08)	4.0( $\pm$ 0.02)	6.0( $\pm$ 0.06) *	9.9( $\pm$ 0.05) **
DB (mg/dl)	0.7( $\pm$ 0.03)	0.8( $\pm$ 0.04)	1.0( $\pm$ 0.01)	2.8( $\pm$ 0.0) *	6.7( $\pm$ 0.02) **
Protein (mg/dl)	6.2( $\pm$ 0.8)	7.4( $\pm$ 0.9) #	7.5( $\pm$ 0.7) #	5.92( $\pm$ 0.4) @	3.6( $\pm$ 0.5) * ###
ALB (mg/dl)	3.3( $\pm$ 0.7)	3.5( $\pm$ 0.6) #	3.2( $\pm$ 0.4)	2.4( $\pm$ 1.0) *	2.0( $\pm$ 0.4) *
<b>Lipid profile</b>					
TG (mg/dl)	102.6( $\pm$ 7.6)	109.3( $\pm$ 7.8)	122.6( $\pm$ 4.6)	190.7( $\pm$ 7.8) *	270.5( $\pm$ 16.2) **
TC (mg/dl)	116.1( $\pm$ 3.4)	121.1( $\pm$ 3.2)	133.2( $\pm$ 4.8)	362.0( $\pm$ 30.8) **	389.2( $\pm$ 24.5) **
LDL (mg/dl)	77.5( $\pm$ 9.5)	88.4( $\pm$ 5.5)	108.8( $\pm$ 7.5)	198.4( $\pm$ 9.6) **	347.2( $\pm$ 26.1) **
VLDL (mg/dl)	21.2( $\pm$ 3.8)	23.1( $\pm$ 3.5)	24.2( $\pm$ 3.5)	41.9( $\pm$ 3.5) *	74.8( $\pm$ 5.9) **

(Table 5) cont....

Parameter	Control	STH-OO	STH-CO	STH-MVO	STH-SO
HDL (mg/dl)	56.1(±5.4)	73.0(±5.3) #	70.4(±6.8) #	35.8(±3.5) \$\$\$	35.1(±1.7) \$\$\$
Tissue damage parameters					
CPK (IU/L)	168.5(±16.0)	201.7(±86.3)	210.1(±15.8)	404.4(±95.6) *	504.4(±95.3) **
LDH (IU/L)	228.5(±19.3)	313.8(±9.9)	352.9(±21.0)	441.6(±23.4) *	641.6(±23.2) **
Homocysteine (µmol/L)	4.6(±0.05)	5.1(±0.17)	5.7(±0.09)	7.3(±0.3) *	8.0(±0.3) **
MDA (nmol/ml)	7.96(±0.31)	7.93(±0.33) #	7.90(±0.5) #	11.0(±0.7) *	12.0(±0.5) **
CRP (mg/dl)	0.142(±0.02)	0.22(±0.04)	0.5(±0.08)	2.5(±0.9) **	2.6(±0.9) **

Data representation (mean ± S.D) \*Values with statistically significant difference ( $p < 0.05$ ) in comparison with control, STH-OO and STH-CO groups; \*\*highly significant difference ( $p < 0.005$ ) in comparison with control, STH-OO and STH-CO groups; # values are more favorable than the control group; @ values with statistically significant difference ( $p < 0.05$ ) than STH-OO and STH-CO only; \$\$\$ highly significant difference ( $p < 0.005$ ) than STH-OO and STH-CO only; \$ Values with statistically significant difference ( $P < 0.05$ ) in comparison with control only

At ( $p < 0.05$ ) values with a statistically significant difference and ( $p < 0.005$ ) values with a statistically highly significant difference

#### 4. DISCUSSION

In this study, we compared and explained the possible health effects of 2 weeks TPN therapy in rabbits, fed on single time heated different oils in a moderate daily dose of 1ml/kg. The outcome and magnitude of TPN-associated adverse effects were different in different oil-fed groups, which supported our hypothesis. Oil consumption for 16 weeks in rabbits justified the long term use of edible oils in humans (equivalent to 12 years of human life), which exerts its effect even after discontinuation of intake [36].

Weight gain was observed in all the groups after 2 weeks of TPN administration to oil fed animals. The highest body and organ weight gain was reported in STH-SO, and STH-MVO fed groups, which is suggestive of induced oxidative stress, damage at the cellular level and poor glycemic and lipid level control. Other groups showed fewer weight variations throughout the study, with the least variations in olive and canola oils. The presence of high Monounsaturated Fatty Acids (MUFA) content in olive and canola oils may explain the resistance of significant weight gain with potent control on blood glucose and lipid profile [37].

The hepatobiliary adverse effects associated with TPN therapy were insignificant in olive and canola oils fed animals. Olive oil with a high content of MUFA and antioxidant polyphenolic compounds exerted hepatoprotective effect again, the TPN induced plasma lipid peroxidation and oxidative stress [38]. With low erucic acid and suitable Linolenic Acid /Alpha-Linolenic Acid (LA/ALNA) ratio in canola oil, it effectively kept the serum total cholesterol and LDL levels lower [39]. The minimal magnitude of TPN induced hepatic toxicity in the olive oil fed group explains the effect of olive oil on maintaining the functional integrity of transaminases and hepatocytes [40]. Furthermore, the results of our study suggest that consumption of single time heated olive and canola oil in the normal recommended daily dose for longer duration may prevent the hepatic damage induced by any hepatotoxic therapeutic agent, like TPN.

On the other hand, in STH-SO and STH-MVO treated groups significantly elevated SGPT, ALP, GGT, and direct bilirubin indicate the induction of TPN associated hepatobiliary

disorders, like cholestasis [41]. These elevated liver enzymes also indicate cellular damage and tissue breakdown that allow the escape of intracellular enzymes from the cytosol into the blood. The decreased serum level of albumin and total protein also supports the TPN associated hepatic toxicity in both the groups [42]. The magnitude of hepatobiliary dysfunction was higher in STH-SO than STH-MVO, which is justified with the high content of PUFA and LA/ALNA ratio in sunflower oil [43]. Moreover, the results of our study show that serum levels of albumin and total protein were consistently higher even after TPN administration in olive and canola oils fed animals, showing the integrality of liver functions in these groups. Our results are consistent with previous studies [40, 44].

TPN therapy is associated with induced hyperlipidemia [45] and oxidative stress [46]. Insignificant changes in lipid profile of STH-OO and STH-CO fed animals, suggest that daily use of olive and canola oils for longer duration may exert resistance to hyperlipidemic effects of therapeutic agents, like TPN. In fact, in olive and canola oils groups, HDL levels were even higher than the control animals. These results are consistent with previous olive and canola oils studies [40, 44].

The significantly increased levels of oxidative stress marker (MDA), tissue damage markers (CPK, LDH, and homocysteine) and inflammatory marker (CRP) in STH-MVO and even higher levels in STH-SO fed animals suggest the effect of sunflower oil. These results recommend that consumption of sunflower oil, alone or in the blended form, may induce degenerative tissue disorders and oxidative stress. A multicenter epidemiologic study supports our results [47]. On the other hand, our results show that the highest resistance against TPN- induced oxidative stress and tissue damage was present in single time heated olive and canola oils fed animals. Results show that MDA levels in STH-OO and STH-CO were even lower than control. This resistance against pathological changes in the olive oil group might be linked with the presence of high content of flavonoids and phenolic antioxidant compounds in olive oil [19]. In canola oil-fed animals, this could be explained with the potential of canola oil to reduce plasma E-selectin by targeting the inflammation and atherogenic pathways, which further resisted the pathological changes induced by TPN administration [39].

#### CONCLUSION

Our study concludes that the type of daily consumed cooking oils exerts a strong effect on the magnitude of outcome

and adverse effects of any therapeutic agents and further suggests the importance of the oil consumption history of any patient before therapy initiation. Especially for therapeutic agents with known adverse effects of hepatotoxicity and dyslipidemia. We also conclude that high PUFA containing oils degrade easily and exert more hepatotoxic effects, especially when used for long-duration, so fail to protect from further injury. On the other hand, high MUFA containing oils like olive and canola oils, are found to have strong resistance against the hepatic injury and lipid peroxidation. Therefore, the oil or the blend of oils used in daily life must contain the most appropriate ratio of different vegetable oils that finally have the potential to resist the adverse effects associated with other therapeutic agents.

#### AUTHORS' CONTRIBUTIONS

1. **G. A.** contributed to the conception or design of the work, conducted the study and wrote the paper. She performed analysis or interpretation of data and edited the paper as per reviewers' comments

2. **A. S.** revised the work critically for important intellectual content, supervised the research, and approved the final version for publishing

3. **K. H.** co-wrote the paper and did final review of the manuscript

4. **S. G. B.** co-wrote the manuscript in the revision phase with PI, edited the paper as per reviewers' comments

All the authors read and approved the final manuscript before submission.

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Research study protocol along with the procedures to be followed during our research study, euthanasia, and ethical authorization of the study all got approved from the Board of Advanced Studies & Research (BASR) of the University of Karachi (BASR number:03296/Pharm).

#### HUMAN AND ANIMAL RIGHTS

No humans were used for studies that are the basis of this research. All animal experiments were conducted in accordance with the Guidelines for euthanasia and ethical authorization of the study, all got approved from the Board of Advanced Studies & Research (BASR) of the University of Karachi (BASR number:03296/Pharm).

#### CONSENT FOR PUBLICATION

Not Applicable

#### AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### FUNDING

None.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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