Original Article

Phytoformulation of *Tagetes patula* for Improving Permeability and Prevention of Hyperlipidaemia

Sneha Nawale[1,*], Sujit Das[2], Priyanka N[2], Ganga Raju M[3]

[1]Department of Pharmacognosy, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana, INDIA.
[2]Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana, INDIA.
[3]Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana, INDIA.

ABSTRACT

The present study was aimed to formulate phytoformulation of *Tagetes patula* (MSTP) and its evaluation for antihyperlipidemic activity with triton X-100 and propyl thiouracil induced hyperlipidaemia models. Microsuspension of *T. patula* extract (50 and 100 mg; MSTP) was developed by precipitation technique. MSTP was assessed for its particle size, pH and drug entrapment efficacy. The results confirmed that microsuspension prepared with *Tagetes patula* extract (MSPT) showed average particle size 5.92 μm, pH 5.04 and 38 % drug entrapment. MSTP was screened for antihyperlipidemic activity with triton X-100 and propyl thiouracil induced hyperlipidaemia models. MSPT at doses 50 and 100 mg/kg; bd.wt,p.o proved a significant ($p<0.01$, $p<0.05$) antihyperlipidaemic potential by reducing levels of TG, TC, VLDL, LDL and shown raised HDL level when results were compared with normal and standard group in propyl thiouracil induced and triton X-100 induced hyperlipidaemia models. MSPT showed prominent antihyperlipidaemic activity as decreased particle size improves the dissolution and bioavailability of poorly soluble secondary metabolites. The results revealed significant antihyperlipidaemic activity for MSPT so can be used for managing hyperlipidaemia and other metabolic disorders.

Key words: *Tagetes patula*, Micro suspension, Triton X-100, PTU, GC-MS.

INTRODUCTION

Dyslipidemia is a condition of elevation in plasma lipids; includes triglycerides (TG), cholesterol, phospholipids along with plasma lipoproteins (VLDL, HDL and LDL).[1] Genetic inheritance and use of high carbohydrate or fat diets may lead to development of dyslipidemia which is widely seen in urban countries. Dyslipidaemia is major sign of atherosclerosis and a variety of cardiovascular diseases (CVS) like coronary heart disease. CVS is one of the major life threatening cause of morbidity and mortality among world-wide population, which contribute to almost one-fourth of the deaths inage group of 25-65 years.[2] Based on Indian Council of Medical Research (ICMR) survey, there is a high dominance of hypercholesterolemia in urban area as compared to rural area.[3] High lipid levels may result due to improved absorption of lipids throughout the gut or enhanced endogenic synthesis of lipids. High fat diet and triton X-100 models are used to induce dyslipidemia.[4-9] Dyslipidemia can be controlled with triton X-100, without change/manipulation in diet, but by blocking the endogenic synthesis of lipid levels or by decreasing fat absorption from guts. Both these elements can be evaluated in normal animals.[10]

Tagetes patula L. (French marigold), Asteraceae was originated in Mexico which is an ornamental plant.[11] Traditionally, *Tagetes patula* was used for its diuretic, antiseptic and insect repellent action.[12] Phytochemical studies of *T. patula*revealed the presence of various active secondary metabolites like flavonoids (5.5%), terpenes, alkaloids, carotenoids, thiophenes and fatty acids.[12-19] *T. patula* flowers were also

Submission Date: 20-12-2019;
Revision Date: 12-05-2020;
Accepted Date: 10-09-2020

DOI: 10.5530/ijper.54.3s.159

Correspondence:
Dr. Sneha Nawale
Associate Profosser, Department of Pharmacognosy, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad-500090, Telangana, INDIA.
Phone: +91-040-3291 2927
E-mail: sneha.nawale11@gmail.com

www.ijper.org

possesses analgesic, anti-inflammatory, insecticidal, antifungal, larvicidal and nematicidal activity.[12,20-25] As variety of active constituents were previously identified from *T. patula* flower head,[26] soxhlation was used to prepare extract of *Tagetes patula* flower by using methanol as solvent. But major drawback of phytoconstituents is poor solubility, poor bioavailability and excess dose.[27] So to overcome the prescribed problem *Tagetes patula* was formulated into micro suspension to increase solubility, thus bioavailability and decrease dose and developed micro suspension of *T. patula* was assessed for its antidyslipidaemic action.

MATERIALS AND METHODS

Preparation of crude extract

Crude plant material as *Tagetes patula* flower heads were obtained from West Godavari district Andhra Pradesh, India. Plant material was authenticated by Dr. P. Suresh Babu, Government Degree College Kukatpally, Hyderabad (TS), India with Voucher Specimen no., TPK-4. The plant material was dried, powdered and used for the soxhlation extraction with methanol.

Identification of active constituents from *Tagetes patula* extract using GC-MS

T. patula extract was analysed for identification of various active constituents by GC-MS instrument (Agilent 6890 series).

Animals

In the present experimental study, animal's wistar rats (170-200 g) were obtained from Gentox biosciences, Hyderabad. IAEC (Institutional Animal Ethical Committee with Reg. No.1175/PO/Re/S/08/CPCSEA) of CPCSEA approved the study protocol of present study.

Phytoformulation (microsuspension) of methanol extract of *Tagetes patula* and its characterization

Microsuspension was used prepared through precipitation method with slight modification.[28] Methanolic extract of *T. patula* (5g) was suspended in ethanol (10 mL) by sonication for 60 sec (solution 1). In order to prepare microsuspension of *Tagetes patula,* chitosan (0.1g), calcium hydroxide (0.5 g) and phosphoric acid (5 mL) were mixed (solution 2). Solutions 1 and solution 2 were agitated at 500 rpm for 1h at room temperature. The reddish opalescent microsuspension (MSPT) was formed.

Organoleptic properties of the MSPT were observed as colour, odour and physical appearance. pH of MSPT was analyzed at 25°C±2°C by pH meter (Elco). Practical yield was calculated using the equation

PY (%) = amount of product obtained/ amount of total solid used (polymer + drug) ×100.

Percent drug loading of MSPT

Specific quantity of MSPT was added in 15 mL of methanol, kept in orbital shaker for 1 hr and filtered through a membrane filter. Resultant solution was analysed spectrophotometrically at 532 nm using a UV spectrophotometer (Shimadzu 1800). The percent drug loading was determined.

Particle size analysis of MSTP

Determination of average particle size of MSPT was carried out by using particle size analyser (W-3275, Nanotrac wave, USA). The particle size was analysed by adding 0.1 mL sample into the viewing unit. The diameter of particles was averaged and expressed as mean ± SD. Dynamic light scattering (DLS) technique was used to find out particle size. DLS analyse the dispersal of particles under Brownian motion and alters its size and its distribution.

In vitro antioxidant assays of MSPT

Free radical scavenging capability of MSTP was evaluated with $HO^{\bullet}$ and $NO^{\bullet}$ radical scavenge assay method reported by Kunchandy and Rao *et al.*[29,30]

In vivo antihyperlipidemic activity

Triton induced hyperlipidemic rat model

Group-I served as normal control, Group-II served as disease control treated with triton X 100 (100 mg/kg b. wt *i.p*), Group-III hyperlipidaemic group treated with MSTP (50 mg/kg b. wt), Group-IV hyperlipidaemic group treated with MSTP (100 mg/kg b.wt.), Group-V hyperlipidaemic rats treated with standard Simvastatin (10 mg/kg b. wt). On 8th day blood was withdrawn through retro-ocular plexus and serum lipid levels were estimated with a cholesterol kit (ERBA diagnostics Mannheim GmbH), the data was analyzed.[31]

Propylthiouracil induced hyperlipidemia model

Group-I served as normal control, Group-II served as disease control group treated with PTU (10 mg/kg b. wt), Group-III hyperlipidaemic group treated with MSTP (50 mg/kg b. wt), Group-IV hyperlipidaemic group treated with MSTP (100 mg/kg b. wt), Group-Vhyperlipidaemic group treated with Simvastatin (10 mg/kg b. wt). On 8th day Group-II to Group-V were treated with cholesterol (400 mg/kg b. wt) and after 6 h, blood was withdrawn through retro-ocular plexus and lipid levels were analyzed.[32]

Histopathology of propylthiouracil induced hyperlipidaemic rats

Experimental animals were sacrificed on 8th day of study protocol, to separated livers. Isolated livers were fixed in formalin (10 %, 24hr) and sent for histopathological study (Varunhisto Path, Hyderabad).

Statistical analysis

Statistical study of experimentation was done with Graphpad Prism 7 software. Analysis was expressed as mean ± SEM, assessed for ANOVA and Dunnett's test (Multiple). Significant differences between treated groups and standard group were considered at $p<0.05$, $p<0.01$.

RESULTS AND DISCUSSION

Identification of phytochemical constituents using GC-MS

Phytochemical investigation of *Tagetes patula* shows the presence of flavonoids, terpenes, triterpenoids, steroids and phenols. Presence of phytoconstituents was confirmed by GC-MS analyser. GC-MS results confirmed the presence of nearly twenty phytoconstituents in crude flower extract. The retention time taken by phytoconstituents of flower head extract varied from 16.02 to 27.35 min.

T. patula methanolic extract showed presence of caryophyllene, hexadecanoic acid, n-hexadecanoic acid, piperitone, terthiophene, heneicosane, vitamin-E, γ-terpinene, docosanoic acid, cyclotetracosane, tetracosanoic acid, α-amyrin, quercetin, sigmasterol with full mass spectrometry.[26]

Antioxidant assay of MSTP

MSTP showed prominent HO˙ and NO˙ radical free radical scavenging capability with *in vitro* HO˙ and NO˙ radical scavenging assay (Table 1).

Formulation and characterization of MSTP

MSTP was prepared using biodegradable polymer, chitosan by homogenization method. Particle size for MSTP was found to be 5.92 ± 0.373 µm (Figure 1). The drug entrapment in MSTP was found to be 38%. The pH of the MSPT was determined at 25°C±2°C by pH meter and pH was found to be 5.04.

MSTPis colloidal dispersions, can be used for release of inadequately water soluble phytoconstituents, which was stabilized by surfactant.[33] With reduced particle size disintegration and dissolution rate and inadequate oral bioavailability of MSTP was improved. The small particle size of MSTP also improves physical stability related to sedimentation.[34]

Furthermore, particle size reduction confers further benefit of elevated mass per volume loading related to high dose.[35] The mean particle size for MSPT was found to be 5.92 µm. Alcohol was used as solvents because of their low dielectric constant (ε value). Lower the ε value higher dissolution capability for hydrophobic drugs. Phosphoric acid was used as antisolvent, which facilitates the precipitation of particles in micro/nano range.

In vivo antihyperlipidaemic activity

Triton X-100 induced hyperlipidaemic rat model

Triton X-100 increases lipid levels in experimental animals while MSTP significantly reduced all determined lipid parameters. MSTP at the doses 50 mg/kg b.wt showed significant reduction in TC ($p<0.001$), TG ($p<0.01$), LDL ($p<0.001$) and VLDL ($p<0.05$) levels and improvement in HDL ($p<0.001$) while comparing with normal control and standard. Also MSTP at 100 mg/kg b.wt significantly decrease TC ($p<0.01$), TG ($p<0.01$), LDL ($p<0.01$) and VLDL ($p<0.01$) and significantly increased HDL ($p<0.01$) in hyperlipidemic animals when compared with control group and standard. Simvastatin significantly reduced lipid parameters TC ($p<0.01$), TG ($p<0.05$), LDL ($p<0.01$) and VLDL ($p<0.01$) except HDL ($p<0.01$) which is significantly increased compared with normal rats. Results were expressed in Table 2.

Results are expressed as Mean ± SEM, ($n=6$), ANOVA followed by Dunnett's test was performed and results were compared with control group # = $p< 0.001$, * = $p<0.05$) and standard (a = $p<0.01$).

Propylthiouracil induced hyperlipidemic rat model

Propylthiouracil and cholesterol magnify the lipid levels in experimental animals while MSTP significantly reduced all determined lipid parameters. MSTP (50 mg/kg b.wt) illustrate significant decreasing in TC ($p<0.01$),

Table 1: *In vitro* hydroxyl radical and nitric oxide radical scavenging assay of MSTP.

S. No.	Test compound	Antioxidant assay	IC_{50} value
1	Ascorbic assay (standard)	Hydroxyl radical scavenging assay	33.2
		Nitric oxide radical scavenging assay	36.4
2	MSTP	Hydroxyl radical scavenging assay	40.1
		Nitric oxide radical scavenging assay	42.5

Table 2:Anti-hyperlipidemic activity for MSTP on Triton X-100 induced hyperlipidaemic rats.

Groups/Treatment	Lipid Profile (mg/dL), Mean ± SEM				
	TC	TG	HDL	LDL	VLDL
Group-I: Normal control	145.5 ± 1.7	85.0 ± 2.1	72.8 ± 1.4	68.6±1.3	6.6 ± 0.3
Group-II: Hyperlipidaemic control	270.6 ± 4.0	231.1±1.8	20.9 ± 0.9	194.1±1.9	49.6± 2.8
Group-III: MSPT (50mg/kg, bd.wt, *p.o*)	181.2 ± 2.1$^{\#, a}$	120.3 ± 1.9$^{\#, a}$	34.7±1.7$^{\#, a}$	124.5±2.6$^{\#, a}$	22.8±1.2$^{*, a}$
Group-IV: MSPT (100 mg/kg, bd.wt, *p.o*)	163.2 ± 2.6$^{*, a}$	102.3 ± 2.7$^{*, a}$	57.6 ±1.7$^{\#, a}$	73.4±3.8$^{*, a}$	17.6±0.5$^{\#, a}$
Group-V: Simvastatin(10 mg/kg, bd.wt, *p.o*)	147.9 ± 2.8$^{\#}$	96.8 ± 2.2*	67.2 ± 2.0$^{\#}$	71.6±4.5$^{\#}$	10.8±0.2$^{\#}$

Table 3: Anti-hyperlipidemic activity for MSPT on Propylthiouracil induced hyperlipidemic rats.

Groups/Treatment	Lipid Profile (mg/dL), Mean ± SEM				
	TC	TG	HDL	LDL	VLDL
Group-I: Normal control	145.5±1.7	85.8±2.04	62.8±1.4	68.6±1.3	15.6±0.3
Group-II: Hyperlipidaemic control	256.9±2.9	223.6±2.9	21.2±1.3	200.3±5.0	44.8±0.5
Group-III: MSPT (50mg/kg, bd.wt, *p.o*)	185.6±3.2$^{\#, a}$	132.9±2.0$^{\#, a}$	27.5±1.2$^{\#, a}$	137.6±2.5 $^{*, a}$	26.7±0.3$^{\#, a}$
Group-IV: MSPT (100 mg/kg, bd.wt, *p.o*)	178.6±2.1$^{*, a}$	114.3±2.5$^{\#, a}$	52.8±1.4$^{*, a}$	104.2±1.5$^{ns, a}$	22.7±0.5$^{\#, a}$
Group-V: Simvastatin (10 mg/kg, bd.wt, *p.o*)	165.9±2.4*	99.9±1.8$^{\#}$	53.7±1.9$^{\#}$	86.7±1.6$^{\#}$	19.8±0.3*

TG (p<0.01), LDL (p<0.05, p<0.01) and VLDL (p<0.01) levels and increasing of HDL (p<0.01) while comparing with normal control and standard. Also MSTP (100 mg/kg b.wt) significantly decreased TC (p<0.05, p<0.01), TG (p<0.01), LDL (p<0.01) and VLDL (p<0.01) and significantly improved HDL (p<0.05) in hyperlipidemic animals, compared with control group and standard. Simvastatin significantly reduced lipid parameters TC (p<0.01), TG (p<0.01), LDL (p<0.01) and VLDL (p<0.05) except HDL (p<0.01) which is significantly enhanced compared with control rats. Results were expressed in Table 3.

Results are expressed as Mean ± SEM, (n=6), ANOVA followed by Dunnett's test was performed and results were compared with control group ($^{\#}$ = p<0.01, * = p<0.05) and standard (a = p<0.01).

Dyslipidaemiais a major risk aspect for various cardiovascular diseases which causes premature death globally and major reason of mortality in India also.[36] As HDL level is inversely related to whole body cholesterol and a decrease in HDL level may enhance the risk of atherosclerosis leading to ischemic heart diseases (IHD) and other CVS diseases[37] by impairing cholesterol clearing from the arterial wall.[38] Triton 100X has normally been used for screening of various drug/herbal products herbs for their hypolipidaemic action.[39] The increased lipid levels (TC, TG and LDL) and decrease in HDL level in Triton X-100 induced hyperlipidaemia have reported earlier.[40,41] As MSPT (50mg/kg bd.wt. and 100mg/kg bd.wt) showed significant antidyslipidaemic activity with triton X 100 and PTU rat models. The reversal in hyperlipidaemic mediate variation in lipid level of experimental animals by administration of MSPT, possibly by restraining HMG-CoA reductase, as that of atorvastatin showed antihyperlipidaemic action.[42,43] This enhanced lipid level might be due to the existence of flavonoids[44] and various polyphenolic compounds present in methanolic extract of *T. patula* which was already confirmed by GC-MS. Antidyslipidaemic activity of MSPT may be due to decreased particle size i.e., 5.92 µm, enhanced surface area, saturation solubility and bioavailability of phytoconstituents.

Furthermore, the antidyslipidaemic action of MSPT was proved with histopathology studies of experimental animal liver tissue. Figure 2 in histopathology of normal rat liver bile duct, kuffer cells, sinusoids and

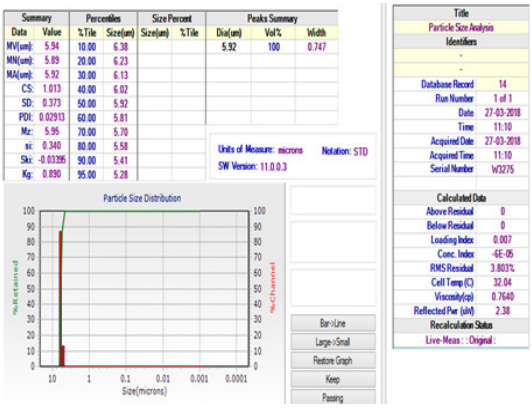


Figure 1: Particle size analysis of MSTP phytoformulation using Nanotrac. Effect of MSTP on lipid levels of propylthiouracil induced hyperlipidaemia

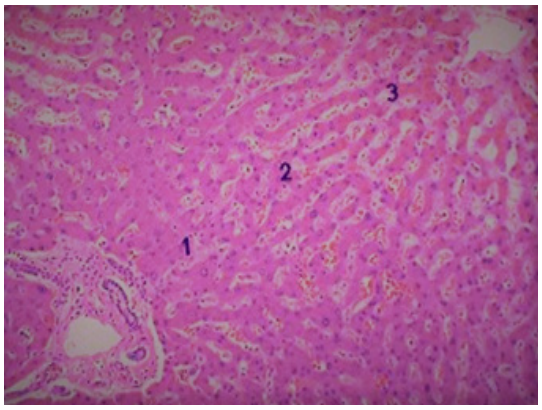


Figure 2: Histopathology of animal liver in control group.

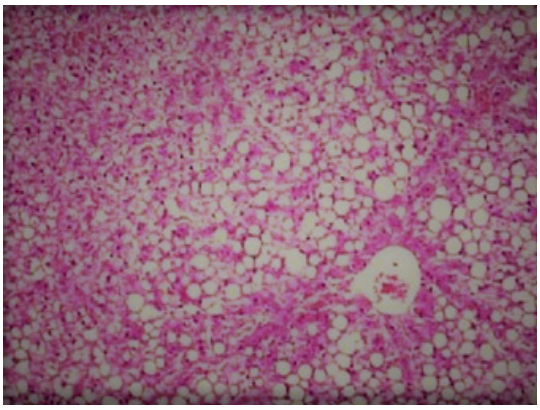


Figure 3: Histopathology of animal liver in hyperlipidaemic control.

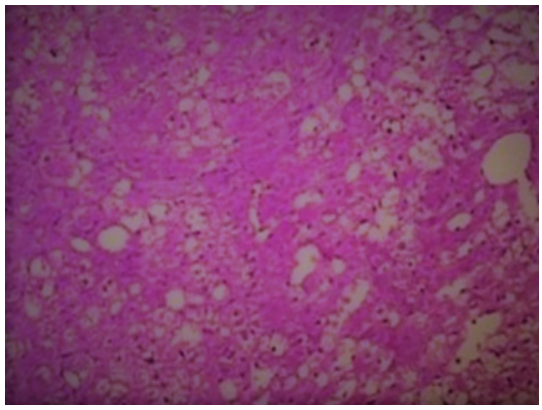


Figure 4: Histopathology of animal liver treated with MSPT 50 mg/kg bd. wt.

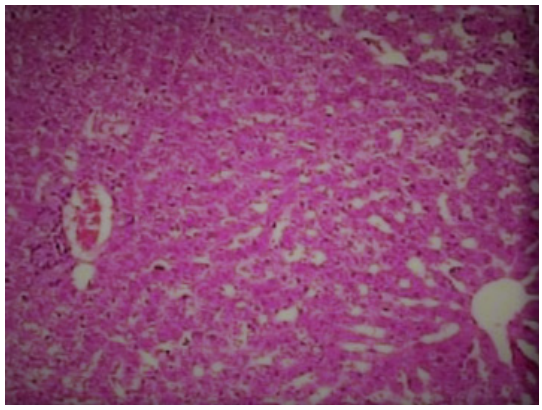


Figure 5: Histopathology of animal liver treated with MSPT 100 mg/kg bd. wt.

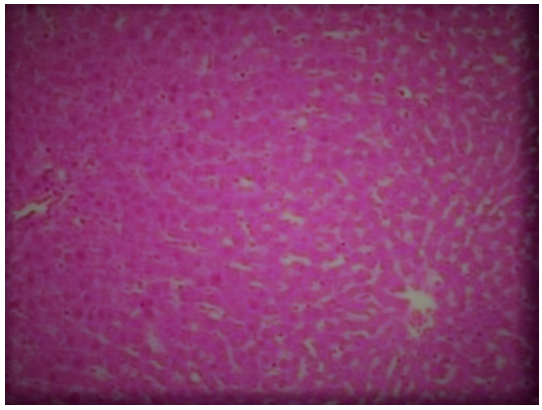


Figure 6: Histopathology of animal liver treated with Simvastatin 10 mg/kg.

fibrosis was observed around the portal area of liver. Figure 3 Histopathology of hyperlipidemic control rat's liver; Cord pattern of hepatocytes, few periportal lymphocytes are noticed in periportal area of liver. Fatty change and fibrosis was observed in cytoplasm. Figure 4 Histopathology of animal liver served with MSPT 50 mg/kg, bd.wt, *p.o*; Moderate sinusoidal space dilatation with haemorrhages was observed in sinusoid region along with few periportal lymphocytes in focal region. Figure 5 Histopathology of animal liver served with MSPT 100 mg/kg, bd.wt, *p.o*; Hepatocytes, periportal and centrilobular region was observed normal, with mild sinusoidal space dilation and hemorrhage. Figure 6 Histopathology of animal liver served with Simvastatin

10 mg/kg, bd.wt, *p.o*); Hepatocytes, periportal lymphocytes, kupffer cells, fibrosis and sinusoids observed normal.

CONCLUSION

Oral dose of MSPT was half to that of extracted dose and exhibited significant antihyper lipidaemic action. As a result we canpropose that microparticle can be used to prevailsolubility issue of inadequately soluble phytoconstituents and to reduce the oral dose. Present study may possibly give a reference for future exploration of phytoformulation as a novel preventive and therapeutic measure for the management of hyperlipidaemia and other metabolic disorders.

ACKNOWLEDGEMENT

Authors acknowledge the support of Gokaraju Rangaraju College of Pharmacy, Hyderabad.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

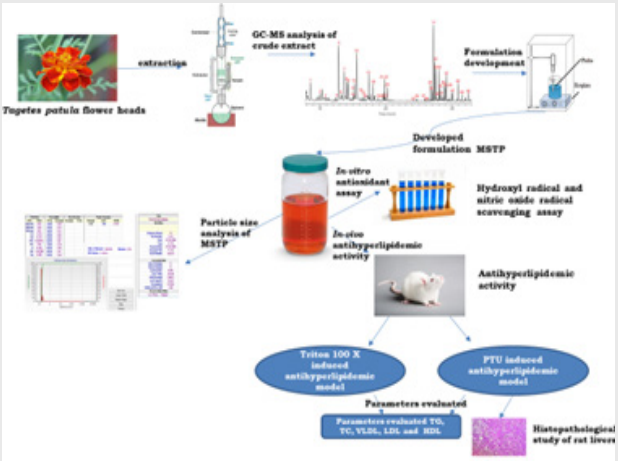
MSTP: Microsuspension of *Tagetes patula*; **GC-MS:** Gas chromatography-mass spectrometry; **PTU:** Propyl thiouracil; **TC:** Total cholesterol; **TG:** Triglycerides; **LDL:** Low density lipoprotein; **VLDL:** Very low-density lipoprotein; **HDL:** High density lipoprotein.

REFERENCES

1. Shattat GF. A Review Article on Hyperlipidemia: Types, Treatments and New Drug Targets. Biomed Pharmacol J. 2014;7(1):399-409.
2. Aslesh OP, Jayasree AK, Karunakaran U, Venugopalan AK, Divakaran B, Mayamol TR, *et al*. Prevalence of hypercholesterolemia among adults aged over 30 years in a rural area of north Kerala, India: A cross-sectional study. WHO South-East Asia J Public Health. 2016;5(1):70-5.
3. Shah B, Mathur P. Surveillance of cardiovascular disease risk factors in India: the need and scope. Indian J Med Res. 2010;132(5):634-42.
4. Rosini TC, Silva AS, Moraes CD. Diet-induced obesity: rodent model for the study of obesity-related disorders. J Brazil Med Assoc. 2012;58(8):383-7.
5. Thirumalai T, Tamilselvan N, David E. Hypolipidemic activity of Piper betel in high fat diet induced hyperlipidemic rat. J Acute Dis. 2014;3(2):131-5.
6. Matos SL, Paula HD, Pedrosa ML, Santos RC, Oliveira EL, Chianca DA, *et al*. Dietary models for inducing hypercholesterolemia in rats. Braz Arch Biol Technol. 2005;48(2):203-9.
7. Girija K, Lakshman K. Anti-hyperlipidemic activity of methanol extracts of three plants of Amaranthus in triton-WR 1339 induced hyperlipidemic rats. Asian Pac J Trop Biomed. 2011;1(1):S62-5.
8. Raj CD, Jayanthi V, Manaswini VS, Gayathri R, Ranjani C, Brindha P. Effect of polyherbal formulation (OB-6) on high fat diet induced hyperlipidemia in rats. Inter J Pharma Pharmaceut Sci. 2012;4(2):31-5.
9. Pasha SR. Evaluation of hypolipidemic activity of methanolic extract of *Ficusdalhousiae*miq. on triton-x and high fat diet induced hyperlipidemia in albino rats. Eur J Pharm Med Res. 2014;1:183-91.
10. Mishra R, Pandit K, Bhagwat AM, Brijesh S. Antihyperlipidemic activity of alcoholic extracts of *Trichosanthesanguina* in triton WR 1339 induced hyperlipidemic rats. Int J Pharm Sci Res. 2014;5(10):4269-74.
11. Vasudevan P, Kashyap S, Sharma S. Tagetes: A multipurpose plant. Bioresour Technol. 1997;62(1-2):29-35.
12. Chadha YR. Tagetes Linn. (Compositae). In The Wealth of India. CSIR: New Delhi.1976;109-12.
13. Prakash O, Rout PK, Chanotiya CS, Misra LN. Composition of essential oil, concrete, absolute and SPME analysis of *Tagetes patula* capitula. Ind Crops Prod. 2012;37(1):195-9.
14. Faizi S, Naz A, Jafrine, A novel and labile β-carboline alkaloid from the flowers of *Tagetes patula*. Tetrahedron.2002;58(31):6185-97.
15. Piccaglia R, Marotti M, Grandi S. Lutein and lutein ester content in different types of *Tagetes patula* and *T. erecta*. Ind Crops Prod. 1998;8(1):45-51.
16. Szarka SZ, Hethelyi E, Lemberkovics E, Kuzovkina IN, Banyai P, Szoke E. GC and GC-MS studies on the essential oil and thiophenes from *Tagetes patula* L. Chromatographia. 2006;63(13):S67-73.
17. Deineka VI, Sorokopudov VN, Deineka LA, Tretyakov MY. Flowers of marigold (Tagetes) species as a source of xanthophylls. Pharm Chem J. 2007;41(10):540-2.
18. Guinot P, Gargadennec A, Valette G, Fruchier AC. Primary flavonoids in marigold dye: extraction, structure and involvement in the dyeing process. Phytochem Anal. 2008;19(1):46-51.
19. Munhoz VM, Longhini R, Silva TAP, Lonni AASG, Souza JRP, Lopes GC, *et al*. Pharmacognostic study of *Tagetes patula* L. (Asteraceae). Rev Fitos. 2012;7:225-30.
20. Faizi S, Dar A, Siddiqi H, Naqvi S, Naz A, Bano SL. Bioassay-guided isolation of antioxidant agents with analgesic properties from flowers of *Tagetes patula*. Pharm Biol. 2011;49(5):516-25.
21. Wells C, Bertsch W, Perich M. Insecticidal volatiles from the marigold plant (genus Tagetes). Effect of species and sample manipulation. Chromatographia. 1993;35(3-4):209-15.
22. Buena AP, Dıez-Rojo MA, Lopez-Perez JA, Robertson L, Escuer M, Bello A. Screening of *Tagetes patula* L. on different populations of Meloidogyne. Crop Prot. 2008;27(1):96-100.
23. Dharmagadda VSS, Naik SN, Mittal PK, Vasudevan P. Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. Bioresour Technol. 2005;96(11):1235-40.
24. Faizi S, Siddiqi H, Bano S, Naz A, Mazhar K, Nasim S, *et al*. Antibacterial and antifungal activities of different parts of *Tagetespatula*: Preparation of patuletin derivatives. Pharm Biol. 2008;46(5):309-20.
25. Yasukawa K, Kasahara Y. Effects of flavonoids from French Marigold (florets of *Tagetes patula* L.) on acute inflammation model. Int J Inflam. 2013;1-5.
26. Sneha NK. Padma PP, Pranusha MGR. Data of antihyerlipidaemic activity for methanolic extract of *Tagetes patula* Linn. Flower head along with piperine, as bioavailability enhancer. Data in Brief. 2018;21:587–97.
27. Kritika KRG. Bioavailability enhancers of herbal origin: An overview. Asian Pac J Trop Biomed. 2013;3(4):253-66.
28. Mohammadpour DN, Eskandari R, Avadi MR, Zolfagharian H, Mir MSA, Rezayat M. Preparation and *in vitro* characterization of chitosan nanoparticles containing *Mesobuthuseupeus* scorpion venom as an antigen delivery system. J Venom Anim Toxins Incl Trop Dis. 2012;18(1):44-52.
29. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharmaceut. 1990;58(3):237-40.
30. Alam M, Bristi N, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm J. 2013;21(2):143-52.
31. Adigun N, OladijiAAT. Antioxidant and anti-hyperlipidemic activity of hydroethanolic seed extract of *Aframomum melegueta* K. Schum in Triton X-100 induced hyperlipidaemicrats. South Afr J Bot. 2016;105:324-32.
32. Adeneye A, Crooks P. Weight losing, antihyperlipidemic and cardioprotective effects of the alkaloid fraction of *Hunteria umbellata* seed extract on normal and triton-induced hyperlipidemicrats. Asian Pac J Trop Biomed. 2015;5(5):387-94.
33. Oberdorster GJ. Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. JInten Med.2010;267(1):89-105.
34. Kim JA, Aberg C, Salvati A, Dawson KA. Role of cell cycle on cellular uptake and dilution of nanoparticles in a cell population. Nature Nanotechnol. 2012;7(1):62-8.

35. Barrett ER. Nanosuspension in drug delivery. Nat Rev Drug Discov. 2004;3(9):785-90.
36. Verlecar XN, Jena KB, Chainy GB. Biochemical markers of oxidative stress in Pernaviridis exposed to mercury and temperature. ChemBiol Interact. 2007;167(3):219-26.
37. Hutcheson R, Rocic P. The metabolic syndrome, oxidative stress, environmentand cardiovascular disease: The great exploration. Exp Diabetes Res. 2012;5:271028.
38. Kanungo SK, Panda DS, Swain SR, Barik BB, Tripathi DK. Comparative evaluation of hypolipidemic activity of some marketed herbal formulations in Triton induced hyperlipidemic rats. Pharmacol Online. 2007;3:211-21.
39. Schurr PE, Schultz JR, Parkinson TM. Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs. Lipids. 1972;7(1):68-74.
40. Gundamaraju R, KimKah H, Singla RK, Vemuri RC, Mulapalli SB. Antihyperlipidemic potential of *Albiziaamara* (Roxb) Boiv. bark against Triton X100 induced hyperlipidemic condition in rats. Pharmacog Res. 2014;6(4):267-73.
41. Thanga K, Kumari S, PackiaLincy M, Muthukumarasamy S, Mohan VR. Antihyperlipidemic effect of ethanol extract of whole plant of *Canscoraperfoliata* Lam in Triton X-100 induced hyperlipidemic rats. Int J Adv Res. 2013;1:166–70.
42. Vijayaraj P, Muthukumar K, Sabarirajan J, Nachiappan V. Antihyperlipidemic activity of *Cassia auriculata* flowers in triton WR 1339 induced hyperlipidemic rats. ExpToxicolPathol. 2011;65(1-2):135–41.
43. Oh PS, Lee SJ, Lim KT. Hypolipidemic and antioxidative effects of the plant glycoprotein (36 kDa) from *Rhusverniciflua* stokes fruit in Triton WR-1339-induced hyperlipidemic mice. Biosci Biotechnol Biochem. 2006;70(2):447-56.
44. Lei G, Wei-Rong H, Ji-Hong L, Wei J, Ting D, Ming Q, Bang-Qiang G. Anti-hyperlipidemic properties of CM108 (a flavone derivative) *in vitro* and *in vivo*. Eur J Pharmacol. 2006;551(1-3):80-6.

PICTORIAL ABSTRACT



SUMMARY

- *Tagetes patula* flowers heads were collected, authenticated and extracted by Soxhlet apparatus with methanol as solvent. Crude methanolic extract obtained was analyzed by GC-MS (Agilent 6890 series) for identification of secondary metabolites.
- Crude methanolic extract was used to develop the phytoformulation (micro suspension) with probe sonicator. Developed formulation (MSTP) was characterized for its particle size with particle size analyzer (W-3275, Nanotrac), pH (Elco) and entrapment efficiency.
- MSTP was evaluated for *in-vitro* antioxidant assay with hydroxyl radical and nitric oxide radical scavenging assay. MSTP was also evaluated for antihyperlipidemic activity with triton 100X and Propyl thiouracil induced hyperlipidaemic models and TC, TG, LDL, VLDL and HDL levels were estimated.
- Results showed that MSTP was having prominent antioxidant activity and significant antihyperlipidemic activity when results were compared with control and standard group. Furthermore, the antidyslipidemic action of MSPT was proved with histopathology studies (Varunhistopath, Hyderabad) of experimental animal liver tissue in PTU induced hyperlipidaemic model.
- Antidyslipidaemic activity of MSPT may be due to decreased particle size i.e., 5.92 μm, enhanced surface area, saturation solubility and bioavailability of phytoconstituents.

About Authors

Dr. Sneha Nawale, working as Associate Professor in Gokaraju Rangaraju College of Pharmacy, Hyderabad. She has 14 years of teaching experience. She has published 41 research articles in various national and international journals. She has attended 20 conferences and presented 12 papers. She has guided 19 M. Pharmacy students and 4 B. Pharmacy students.

Sujit Kumar Das completed Master in Pharmacology from Gokaraju Rangaraju College of Pharmacy, Hyderabad, presented 5 poster. Now perusing PG public health from Capebreton University, Sydney, Canada.

Priyanka N. completed Master in Pharmacology from Gokaraju Rangaraju College of Pharmacy, Hyderabad, presented 6 papers as posters and has 2 research publications her credit.

Prof. M. Ganga Raju, Vice Principal and Head of the Department, Gokaraju Rangaraju college of Pharmacy, Hyderabad. He has 17 years of teaching experience in Osmania Affiliated College. He has guided 25 M. Pharmacy students. To his credit he has more than 50 International publications in peer reviewed journals. He has presented more than 15 papers in various national and international conferences. He was the Vice President, Indian Pharmacological Society, local chapter, Telangana from January 2017 to till date.

Cite this article: Nawale S, Das S, Priyanka N, Raju GM. Phytoformulation of *Tagetes patula* for Improving Permeability and Prevention of Hyperlipidaemia. Indian J of Pharmaceutical Education and Research. 2020;54(3s):s593-s600.