International Journal of Clinical Trials
Aslam MN et al. Int J Clin Trials. 2020 Aug;7(3):170-175
http://www.ijclinicaltrials.com
pISSN 2349-3240 | eISSN 2349-3259
DOI: http://dx.doi.org/10.18203/2349-3259.ijct20203103

Original Research Article

Efficacy and safety of *Prunus mume* and choline in patients with abnormal level of liver function test

Muhammad N. Aslam[1], Anwar Ali[2], Suresh Kumar[3]*

[1]Department of Gastroenterology, Mayo Hospital, Lahore, Pakistan
[2]Department of Medicine, Saidu Group of Teaching Hospital, Khyber Pakhtunkhwa, Pakistan
[3]Department of Medicine, Rafa-e-Aam Hospital, Sindh, Pakistan

Received: 18 March 2020
Revised: 27 April 2020
Accepted: 30 April 2020

***Correspondence:**
Dr. Suresh Kumar,
E-mail: sureshkotwanti@gmail.com

ABSTRACT

Background: The objectives of the study were to determine the efficacy and safety of *Prunus mume* and choline in patients with abnormal liver function test.
Methods: This open labelled, multi-centered observational study was done for a from May 2019 to December 2019. Patients of either gender, above 18 years of age having elevated levels of aspartate aminotransferase, alanine transaminase or gamma-glutamyltransferase were included in the study after taking informed consent. One to two tablets of revolic per day were given preferably in the morning with breakfast or as per instructions of the physician. Patient follow-ups were done at 2nd and 4th week after treatment. SPSS version 20.0 was used for analysis. Frequency and percentages and for quantitative data, mean, standard deviation, median and interquartile range were recorded. Wilcoxon signed ranks test was applied with p value of <0.05 as significant level.
Results: Among 247 patients, male to female ratio was 2:1 with overall mean age of 42.8±12.6 years. Total bilirubin decreased from baseline to week-4 in treatment with *Prunus mume* extract and choline (p=0.04). Median and IQR of alanine transaminase levels also reduced substantially from 99 (52) to 42 (36.5) I/U. Aspartate aminotransferase levels significantly decreased from 78.5 (57) to 40 (29) I/U. Overall at the end of treatment on week-4, 26 (10.5%) patients experienced gastrointestinal distress, 25 (10.1%) anorexia, 14 (5.7%) excessive salivation and 29 (11.7%) patients experienced excessive sweating.
Conclusions: This study reported significant improvement in alanine transaminase and aspartate aminotransferase levels after treatment with *Prunus mume* extract and choline (revolic). It is safe and effective to use them for deranged liver functions tests.

Keywords: *Prunus mume* extract, Choline, Liver function tests

INTRODUCTION

Among various hepatic disorders, the most commonly observed liver diseases are non-alcoholic fatty liver disease, steato-hepatitis and hepatocellular carcinoma.[1] Even though multiple causes of liver diseases have been associated with it, infection with hepatitis B and hepatitis C especially in under-developed and developing countries is highest. In developed world, alcohol is the leading cause of chronic liver disease.[2] Other lifestyle-associated risk factors include diabetes, obesity, smoking and non-alcoholic steato-hepatitis. Although substantial improvements have been made in treatment modalities through medications, yet the mortality rates have not shown significant improvements and so therefore, newer therapeutic strategies are the utmost need for putting an end to such devastating disease.[3]

Until recently, oxidative stress was reported to be one of the main risk factors for liver disease.[4] Many herbal extracts having anti-oxidant activity like allium sativum, glycyrrhizaglabra, taxacumofficinale, silybummarianum and cichoriumintybus, all are studies for the beneficial effect on a variety of diseases including hepatic disorders.[5] *Prunus mume*, originating from south-eastern China, is known as an anti-inflammatory and anti-oxidative fruit. Extract of *Prunus mume* is an herbal variant that has been reported to exert a potentially therapeutic effect for various conditions such as inflammatory bowel disease, diabetes mellitus, vascular dementia and cancers, including hepato-cellular carcinoma.[6]

Prunus mume is regarded to contain 5-O-caffeoylquinic acidand 3-O-caffeoylquinic acid, both found to be a major bioactive anti-oxidant compound.[7] *Prunus mume* has been observed to be an effective treatment modality in alcoholic liver disease having elevations in serum as well as hepatic triglycerides where serum triglycerides were reported to reduce in treatment with *Prunus mume*. It could be used for preventing alcoholic liver steatosis. Additionally, *Prunus mume* was seen to reduce cellular oxidative stress through inhibition of mitogen-activated protein kinase's activation and p-53 mediated apoptotic axis of signaling. This resulted in inhibiting hepatic steatosis and apoptosis as well.[8]

The constituents of *Prunus mume*, also known as Japanese apricot included triterpenoids, like oleanolic acid and ursolic acid, which has reported to inhibit cellular growth and induce multiple tumors' cell death, such as in gastric cancer, pro-myelocytic leukemia, pancreatic cancer, breast cancer, colon cancer, malignant myeloma, esophageal cancer, lung cancer and hepato-cellular cancer.[9] Lately, the hepato-protective effects of *Prunus mume* have been observed in patients among chronic liver disease patients.[10]

Since choline is used in synthesis of acetylcholine, a neurotransmitter as well as involved in methyl-group metabolism, especially in the liver. Therefore, the major site of choline metabolism is the liver, were it is mainly found as phosphatidylcholine.[11] It is associated with assembly / secretion of lipoproteins and to solubilize cholesterol in bile. The link between choline deficiency and accumulation of hepatic lipid has been recognized for over 50 years, leading to the fact that choline-deficient diets ought to induce non-alcoholic fatty liver disease. Little is known regarding choline's role in potentially preventing or in treating non-alcoholic fatty liver disease.[12,13]

Choline is a content of cell membrane, mitochondrial membrane and also an important component of neurotransmitter, acetylcholine. Due to its essential ubiquitous presence in cellular components and pathways, it is regarded as a main nutrient in metabolism and through phosphorylation are used to producing phospholipids or are oxidized and used as methyl group donor.[14] The hepatic metabolite of choline is phosphatidylcholine, which is essentially important for packaging, export of triglycerides in very low density lipoprotein and for bile salt solubility to be secreted.[15]

Multiple studies have been conducted outside the country but there is little data in Pakistan to support for the effectiveness of *Prunus mume* and choline. Therefore, the objective of this study was to determine the safety and efficacy of *Prunus mume* and choline in patients with abnormal level of liver function tests.

METHODS

This open labeled, multi-centered observational study was done for a period of 06 months from May 2019 to December 2019 by using convenient sampling technique. The study was conducted in Mayo Hospital, Lahore, Saidu Group of Teaching Hospital, Swat and Rafa-e-Aam Hospital, Karachi. Using openEpi software, the sample size calculator version 3.01 was used and using 47% efficacy of drug, at 07% margin of error and 95% confidence interval, a sample size of 196 was calculated. Total of 247 patients were included in the study.[16]

After taking written informed consent and the study will be carried out in compliance with the protocols and the principles of the Declaration of Helsinki, and in accordance with the International Conference on Harmonization harmonized tripartite guideline for good clinical practice. Patients of either gender, above 18 years of age having elevated levels of aspartate aminotransferase (AST), alanine transaminase (ALT) or gamma-glutamyl transferase (GGT), were included in the study. Patients with obstructive jaundice, pregnant and lactating mothers, having presence of underlying chronic renal failure were excluded from the study.

Patients were given one to two tablets revolic per day, preferably in the morning with breakfast or as per instructions of the physician. The changes of serum ALT and AST at baseline, 2nd week and 04th week of treatment were noted after treatment with *Prunus mume* and choline. Similarly, changes in (GGT) were also noted. The proportion of patients experiencing any adverse event such as gastrointestinal distress, anorexia, salivation and sweating after taking the tablets were noted and reported. These study parameters and sample size was selected in accordance with the study performed by Beretta et al in 2016.[16]

SPSS version 20.0 was used for analysis of data. For qualitative data, frequency in percentages was reported and for quantitative data, mean, standard deviation, median and interquartile range was recorded. Wilcoxon signed ranks test was applied to test for significant difference at baselineweek-02 and week-04 keeping p-value of <0.05 as statistically significant.

RESULTS

In a total of 247 patients, 170 (69.1%) male and 77 (30.9%) were included in the study. Patients were divided into age groups with most common age group of above 50 years with the frequency of 68 (27.5%). Mean age of patients was 42.8±12.6 years and weight were 78.1±18.4 kg. 16 (8%) patients were diagnosed as hepatitis A, 08 (4%) as hepatitis B, 23 (11.6%) as hepatitis C, 11 (5.6%) as non-specific hepatitis, 5 (2.5%) as auto-immune hepatitis and 135 (68%) as non-alcoholic fatty liver disease. 44 (18%) patients were diabetic, 55 (22%) hypertensive, 64 (26%) having hyperlipidemia, 57 (23%) and 78 (32%) obesity (Table 1).

The median and IQR (inter-quartile range) of total bilirubin was 1.1(2.3) mg/dl at week-2 (p=0.04). ALT was 99 (52) I/U at baseline, 61 (43) mg/dl at week-2 (p<0.01) and 42 (36.5) mg/dl at week-4 (p<0.01). AST was 78.50 (57) I/U at baseline, 50 (32) mg/dl at week-2 (p<0.01) and 40(36.5) mg/dl at week-4 (p<0.01). GGT was 40 (100.5) I/U at baseline, 18 (06) mg/dl at week-2 (p=0.31) and 30 (97.5) mg/dl at week-4 (p=0.12) (Table 2, Figure 1).

Overall, the end of treatment on week-4, 26 (10.5%) patients experienced gastrointestinal distress, 25 (10.1%) anorexia, 14 (5.7%) excessive salivation and 29 (11.7%) patients experienced excessive sweating.

Table 1: Baseline characteristics of studied samples (n=247).

Characteristics		N	%
Gender	Male	170	69.1
	Female	77	30.9
Age group (in years)	16-30	56	22.6
	31-40	57	23.0
	41-50	66	26.7
	>50	68	27.5
	Mean±SD	42.8±12.6	
Weight (kg)	Mean±SD	78.1±18.4	
Diagnosis	Hepatitis A	16	8.1
	Hepatitis B	8	4.0
	Hepatitis C	23	11.6
	Nonspecific hepatitis	11	5.6
	Autoimmune hepatitis	5	2.5
	NAFLD	135	68.2
Co-morbidities	Diabetes	44	17.8
	High blood pressure	55	22.3
	High cholesterol	64	25.9
	Smoking	57	23.1
	Obesity	78	31.6

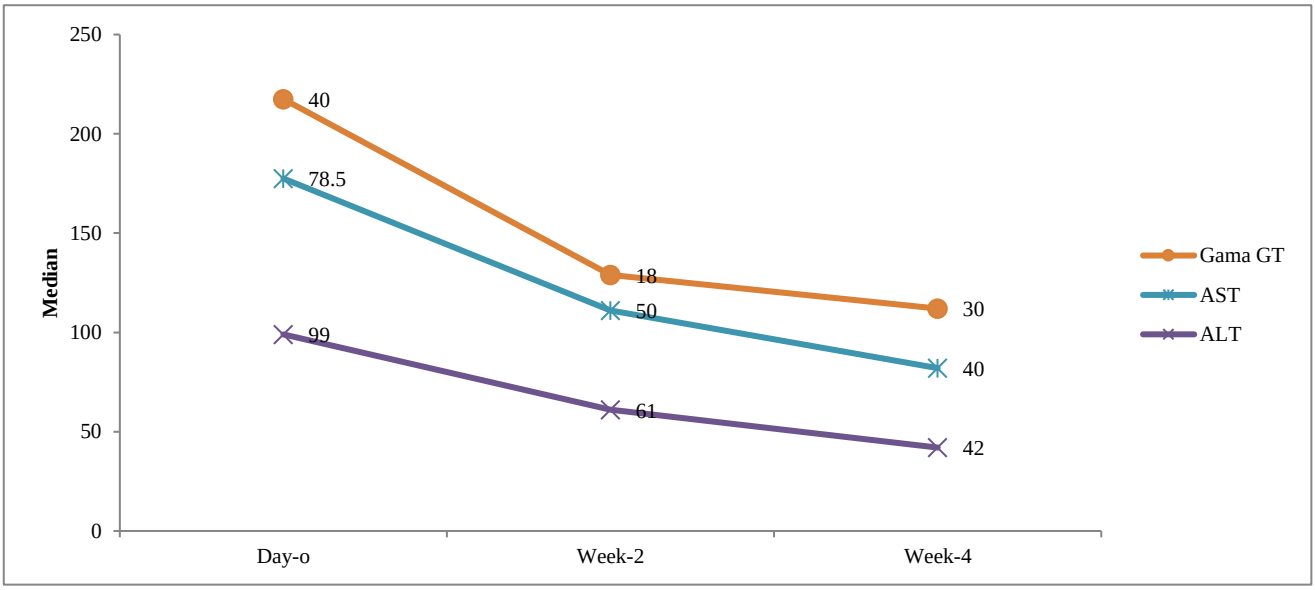

Figure 1: The level of ALT, AST and gamma GT at baseline, 2nd and 4th week.

Table 2: Median comparison of parameters from baseline to week 4.

Parameters	Baseline		Week 2		Week 4		P value	
	Median	IQR	Median	IQR	Median	IQR	Baselines week 2	Baselines week4
Total bilirubin	0.75	0.8	1.1	2.3	0.79	0.4	0.04*	0.11
Direct bilirubin	0.24	0.1	0.7	0.9	0.24	0.1	0.18	1.0
Indirect bilirubin	0.40	0.28	0.3	0.3	0.50	0.3	0.31	0.70
ALT	99.0	52	61.0	43.0	42.0	36.5	<0.01*	<0.01*
AST	78.50	57	50.0	32.0	40.0	29.0	<0.01*	<0.01*
Gama GT	40.0	100.5	18.0	6.0	30.0	97.5	0.31	0.12

*p<0.05 was considered significant using Wilcoxon signed ranks test.

DISCUSSION

In addition to hepato-protective effects, *Prunus mume* has been reported to have anti-inflammatory and anti-oxidant affects both in-vitro as well as in-vivo.[17] In studies where induced hepatotoxicity having high ALT and AST levels, *Prunus mume* was reported to substantially reduce lipid peroxidation and levels of malondialdehyde within liver tissues, thereby reducing the enzymatic levels of ALT and AST.[18] *Prunus mume* is reported to exert hepato-protective effect in both acute, induced liver injury as well as in chronic hepatic fibrosis and cirrhosis. The proposed pathway by which *Prunus mume* works is attributed to enzymatic suppression, leading to reduction in hepatic damage as well as increasing anti-oxidant chemicals which exert an even more hepato-protective effect on liver mitochondria.[19]

Similarly, choline is regarded as an essential nutrient since studies have reported choline as an indispensable nutrient where human with deprivation of choline were seen to develop either fatty liver disease and induce hepatic cell death and also found to have significant skeletal muscle damage.[20] Clinical evidences have reported that patients who are on total parenteral nutritional support having low choline was found to develop fatty liver as well as liver damage. Researches have reported that there exists two main source of human dietary requirement of choline: status of estrogen and variations in genetics.[21] Through endogenous biosynthesis of hepatic phosphatidylcholine, dietary requirement of choline can be spared. Gene for phosphatidylcholine synthesis is expressed and induced by estrogen therefore in pre-menopausal and post-menopausal women treated with estrogen; dietary requirements of choline are diminished. On the other hand, some females have genetic polymorphism that makes them unresponsive to estrogen and they require higher levels of choline daily, similar to men.[22]

The results of our study also reported similar findings to both *Prunus mume* and choline as reported in the literature, where significant decrease in levels of total bilirubin (p=0.04), ALT (p<0.01) and AST were reported (p<0.01) were reported at both baseline and week 4. With regards to the observed side effects, majority of the patients were free of side effects while the patients who experienced side effects, they were more or less tolerable. 11.7% experienced sweating, 10.5% GI upset, 10% anorexia while 5.7% salivation.

In line with our study, the hepato-protective effect of PM among 58 patients with liver disease of hepatitis C, non-alcoholic fatty liver disease and auto-immune liver disease reported that after 12 weeks of treatment, serum ALT and AST levels dramatically decreased in comparison with baseline levels.[18]

Similarly, Nagi et al Inappropriate, reported can be used and only reported in their study on 49 mice divided into 07 groups reported AST of 78±2.64 I/U and ALT of 126±1.00 I/U at baseline was reduced to 54±2.64 I/U and 86±3.60 I/U respectively, after treatment with PM extracted from dried apricots.[23]

Prunus mume has reported to have documented evidence of improving liver function either in alcoholic, non-alcoholic liver disease, hepatic fibro-proliferative disease, chronic hepatitis and overall damaged hepatic health.[24] Its activity involves hepato-protective activity, anti-oxidant/ anti-inflammatory activity, increasing HDL cholesterol as well as improving glycemic levels, thereby elevating the overall function of liver and preventing metabolic and inflammatory-based disorders.[25]

Several mouse models have reported choline administration in non-alcoholic fatty liver disease have reported significant improvements in liver function status.[26,27] Low choline is vital in non-alcoholic fatty liver disease pathophysiology since it can disturb mitochondrial functioning as well as fatty acid oxidation. Deficiency of choline causes alterations in mitochondrial composition and decreases breakdown products of choline thereby reducing ATP production in mitochondria of rate given choline deficient diets.[28] Similarly, in our study, a significant improvement was observed when choline was given to the patients having altered levels of liver function tests.

Limitations of the study

Although this study evaluated substantial data regarding safety and efficacy of *Prunus mume* and choline, however the study was not immune from selection and observer bias. Due to the fact that they study was conducted on a limited sample size, therefore further larger studies are needed to evaluate the safety and efficacy of *Prunus mume* and choline on altered ALT and AST levels.

CONCLUSION

This study reported significant improvement in alanine transaminase, aspartate aminotransferase and gamma-glutamyl transferase levels after treatment with *Prunus mume* and choline (revolic). The most common side effect that was observed was gastrointestinal disturbance of minor nature. Therefore, it is safe and effective to use them for abnormal liver functions tests.

ACKNOWLEDGEMENTS

We are thankful to Muhammad Shakil for providing logistic support of this research project.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84.
2. Younossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. Clin Gastroenterol Hepatol. 2011;9(6):524-30.
3. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328-57.
4. Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol. 2014;20(25):8082-91.
5. Hassan HA, El-Gharib NE, Azhari AF. Role of natural antioxidants in the therapeutic management of hepatocellular carcinoma. Hepatoma Res. 2016;2:216-3.
6. Mitani T, Horinishi A, Kishida K. Phenolics profile of mume, Japanese apricot (Prunus mume Sieb. etZucc.) fruit. Biosci Biotechn olBiochem 2013;77:1623.
7. Yan XT, Lee SH, Li W. Evaluation of the antioxidant and anti-osteoporosis activities of chemical constituents of the fruits of Prunusmume. Food Chem 2014;156:408-15.
8. Khan A, Pan JH, Cho S, Lee S, Kim YJ, Park YH. Investigation of the hepatoprotective effect of PrunusmumeSieb. etZucc extract in a mouse model of alcoholic liver injury through high-resolution metabolomics. J Med Food. 2017;20(8):734-43.
9. Zhang Q, Chen W, Sun L, Zhao F, Huang B, Yang W, et al. The genome of Prunusmume. Nature Communications. 2012;3:1318-26.
10. Jin HL, Lee BR, Lim KJ, Debnath T, Shin HM, Lim BO. Anti-inflammatory effects of Prunusmume mixture in colitis induced by dextran sodium sulfate. Korean J Med Crop Sci. 2011;19(1):16-23.
11. Sherriff JL, O'Sullivan TA, Properzi C, Oddo JL, Adams LA. Choline, its potential role in nonalcoholic fatty liver disease, and the case for human and bacterial genes. Adv Nutr. 2016;7(1):5-13.
12. Mokhtari Z, Gibson DL, Hekmatdoost A. Nonalcoholic fatty liver disease, the gut microbiome, and diet. Adv Nutr. 2017;8(2):240-52.
13. Perumpail BJ, Cholankeril R, Yoo ER, Kim D, Ahmed A. An overview of dietary interventions and strategies to optimize the management of non-alcoholic fatty liver disease. Diseases. 2017;5(4):23-34.
14. B Hollenbeck C. An introduction to the nutrition and metabolism of choline. Central Nervous System Agents Med Chem. 2012;12(2):100-13.
15. Fagone P, Jackowski S. Phosphatidylcholine and the CDP-choline cycle. Biochim Biophys Acta. 2013;1831(3):523-32.
16. Beretta A, Accinni R, Dellanoce C, Tonini A, Cardot JM, Bussière A. Efficacy of a Standardized Extract of Prunus mume in Liver Protection and Redox Homeostasis: A Randomized, Double-Blind, Placebo-Controlled Study. Phytotherapy Res. 2016;30(6):949-55.
17. Xia D, Shi J, Gong J, Wu X, Yang Q, Zhang Y. Antioxidant activity of Chinese mei (Prunusmume) and its active phytochemicals. J Med Plants Res. 2010;4(12):1156-60.
18. Hokari A, Ishikawa T, Tajiri H, Matsuda T, Ishii O, Matsumoto N, et al. Efficacy of MK615 for the treatment of patients with liver disorders. World J Gastroenterol. 2012;18(31):4118-26.
19. Yan XT, Lee SH, Li W, Sun YN, Yang SY, Jang HD, et al. Evaluation of the antioxidant and anti-osteoporosis activities of chemical constituents of the fruits of Prunusmume. Food Chem. 2014;156:408-15.
20. Caballero F, Fernandez A, Matias N. Specific contribution of methionine and choline in nutritional nonalcoholicsteatohepatitis: impact on mitochondrial S-adenosyl-L-methionine and glutathione. J Biol Chem. 2010;285:18528-36.

21. Spencer MD, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA. Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. Gastroenterology. 2011;140(3):976-86.
22. Yu D, Shu XO, Xiang YB, Li H, Yang G, Gao YT, et al. Higher dietary choline intake is associated with lower risk of nonalcoholic fatty liver in normal-weight Chinese women. J Nutr. 2014;144(12):2034-40.
23. Nagi HM, Amin WS, Zaki SA. The potential effect of fruits and vegetables on liver functions and liver alterations induced by acrylamide in mice. InProceedings of 3rd International Conference on Nutrition and Food Sciences (ICNFS 2014), Singapore: IACSIT Press. 2014;71:5-9.
24. Khan A, Pan JH, Cho S, Lee S, Kim YJ, Park YH. Investigation of the hepatoprotective effect of PrunusmumeSieb. etZucc extract in a mouse model of alcoholic liver injury through high-resolution metabolomics. J Med Food. 2017;20(8):734-43.
25. Beretta A, Accinni R, Dellanoce C, Tonini A, Cardot JM, Bussière A. Efficacy of a Standardized Extract of Prunusmume in Liver Protection and Redox Homeostasis: A Randomized, Double-Blind, Placebo-Controlled Study. Phytotherap Res. 2016;30(6):949-55.
26. Schugar RC, Huang X, Moll AR, Brunt EM, Crawford PA. Role of choline deficiency in the Fatty liver phenotype of mice fed a low protein, very low carbohydrate ketogenic diet. PloS one. 2013;8(8):74806-21.
27. Guerrerio AL, Colvin RM, Schwartz AK, Molleston JP, Murray KF, Diehl A, et al. Choline intake in a large cohort of patients with nonalcoholic fatty liver disease. Am J Clin Nutr. 2012;95(4):892-900.
28. Ueland PM. Choline and betaine in health and disease. J Inherited Metabol Dis. 2011;34(1):3-15.

Cite this article as: Aslam MN, Ali A, Kumar S. Efficacy and safety of *Prunus mume* and choline in patients with abnormal level of liver function test. Int J Clin Trials 2020;7(3):170-5.