ORIGINAL RESEARCH ARTICLE

Current Research on Biosciences and Biotechnology **4** (1) 2022 246-250

Current Research on Biosciences and Biotechnology

www.crbb-journal.com

Antihypertensive activity and acute toxicity of turmeric (*Curcuma longa* L.) in L-NAME-induced hypertension animals

Patonah Hasimu*, Yani Mulyani, Irma Yulianti

Faculty of Pharmacy, Bhakti Kencana University, Bandung, West Java, Indonesia

ABSTRACT

Hypertension is one of the significant risk factors for cardiovascular disease and can lead to complications. Uncontrolled hypertension can lead to vascular endothelial dysfunction and other complications of cardiovascular disease. This study aimed to determine the effect of turmeric rhizome (Curcuma longa L.) in extracts and the fractions on a hypertensive rat's model induced by L-NAME 40 mg/kg for three weeks and an acute toxicity study of the extract. Antihypertensive research was performed on male Wistar rats utilizing non-invasive procedures. Turmeric extract at doses of 50, 100, and 200 mg/kg and its fraction of n-hexane, acetyl acetate, and ethanol at a dose of 25 mg/kg, respectively, were given daily per oral for three weeks to 2.5 mg/kg captopril. The systolic and diastolic blood pressure were measured at 0, 21, and 42 days after treatment and was calculated as mean arterial blood pressure (MAP). Acute toxicity testing refers to the OECD 420 Fixed-Dose method with several dosage levels, consists of 300, 2000, and 5000 mg/kg. The turmeric extract at doses of 50, 100, and 200 mg/kg and its fraction of n-hexane, acetyl acetate, and ethanol significantly reduced mean arterial blood pressure ($p<0.05$) compared to the control group. Acute administration of turmeric extracts up to a dose of 5000 mg/kg in test animals did not show any death. Turmeric and its components are considered to possess antihypertensive actions. Antihypertensive activity increased in a dose-dependent manner. Turmeric extract is categorized as being almost entirely non-toxic.

Article history:

Received 02 Aug 2022
Revised 26 Aug 2022
Accepted 29 Aug 2022
Available online 31 Aug 2022

Keywords:

Acute toxicity
Curcuma longa L.
hypertension
L-NAME
mean arterial pressure

*Corresponding authors:
patonah@bku.ac.id

DOI: 10.5614/crbb.2022.4.1/B2AMBRI1

e-ISSN 2686-1623/

1. Introduction

Cardiovascular disease is the leading cause of death worldwide. Hypertension is a significant risk factor for cardiovascular disease and can result in problems of vital organs if therapeutic goals are not met (Mozaffarian et al., 2015). Uncontrolled hypertension can result in cardiovascular problems such as myocardial infarction, heart failure, stroke, peripheral artery disease, and chronic kidney disease (Baradaran et al., 2014). If left untreated, this results in vascular dysfunction with increased endothelial cell permeability. It causes edema and inhibits the synthesis of nitric oxide (NO), and has a pro-inflammatory impact on vascular smooth muscle cells. As a result, oxidative stress is induced, resulting in complications in various vital organs (Zhang et al., 2020).

L-nitro arginine methyl ester (L-NAME) is an L-arginine analogue that acts as an inhibitor of the endothelial nitric oxide synthase (eNOS) enzyme, hence decreasing nitric oxide (NO) production and resulting in systemic vasoconstriction and vascular resistance (Maneesai et al., 2016; Nakmareong et al., 2011). Thus, L-NAME can generate a rise in blood pressure to create an animal model of arterial stiffness-induced hypertension.

Turmeric or *Curcuma longa* L. has been known to have various health benefits, including hypertension. Among the curcuma species, *Curcuma longa* L. has the highest number of curcuminoids (Li et al., 2011). There are three main components in curcuminoids, including curcumin, desmethoxycurcumin, and bisdemethoxycurcumin (Inoue et al., 2008). Previous clinical studies reported that the administration of turmeric extract in combination with garlic extract could improve lipid profiles and control blood glucose levels in hyperlipidemic patients with diabetes mellitus (Sukandar et al., 2010). Also, treatment with curcumin can improve aortic stiffness in mice (Nakmareong et al., 2011; Nakmareong et al., 2012). Additionally, *Centella asiatica* and *Curcuma longa* reduced blood pressure and enhanced arterial stiffness (Hasimun et al., 2019).

Thus, it is crucial to ensure its safety, as any use of turmeric cannot be determined to be safe or hazardous, particularly in vital organs such as the heart, liver, and kidneys. The purpose of this study was to determine the effect of turmeric (*Curcuma longa* L.) rhizome extracts on L-NAME-induced hypertensive rat model and conduct an acute toxicity assessment. The antihypertensive activity of fractionated turmeric, which was separated based on its polarity, was also examined in this study.

2. Materials and methods

2.1. Plant material preparation

The extract of turmeric rhizome (*Curcuma longa* Linn) used in this study was obtained from the Indonesian Spice and Medicinal Research Institute (Balitro) in Bogor, Indonesia. The solvent used for extraction was 70% ethanol. In the Laboratory of the Center for Plant Conservation and Botanical Garden Research-LIPI, the plant specimens were identified and authenticated (No. B-3896/IPH.3/KS/XI/2019). Three solvents with varying polarity

were used to separate the turmeric extract: n-hexane, ethyl acetate, and 70% ethanol.

2.2. Chemicals and drugs

The N-nitro L-arginine methyl ester (L-NAME) was obtained from Sigma (product number N5751), and captopril was obtained from a local pharmacy as a product of Errata Pharma, Indonesia.

2.3. Animal preparation

Wistar rats aged 2-3 months and weighing between 150-200 g were used in this study. The test animals were housed in a laboratory animal cage for seven days and given regular rat food and water ad libitum. A 12-hour dark-light cycle was maintained in the laboratory. All treatment protocols comply with the guidelines for the care of experimental animals and have been approved by the Medical Faculty's Ethics Committee at Padjajaran University, Bandung, West Java, Indonesia. (No. 246/UN6.KEP/EC/2020 for acute toxicity study, and No. 347/UN6.KEP/EC/2020 for antihypertensive study).

2.4. Antihypertensive study design

Thirty male Wistar rats were randomly divided into six groups: group 1 was designated as the normal group (receiving drug carriers), group 2 was defined as the positive group (receiving drug carriers), group 3 was assigned as the comparison group (receiving captopril at a dose of 2.5 mg/kg), and groups 4-6 received turmeric extract at doses of 50, 100, 200 mg/kg. L-NAME 40 mg/kg was administered to all groups except the normal group for 21 days. Furthermore, the drug is administered daily from days 22 to 42. Using a CODA (Kent Scientific Corporation) instrument, the systolic and diastolic blood pressures were assessed non-invasively on days 0, 21, and 42. The mean arterial pressure (MAP) and standard deviation were calculated. The MAP is equivalent to one-third of the difference between the systolic and diastolic pressures (Meaney et al., 2000).

The experimental design for determining the antihypertensive activity of the turmeric fraction was similar to that previously mentioned. The dosage of each fraction was 25 mg/kg for the n-hexane, ethyl acetate, and ethanol fractions.

2.5. Acute toxicity test method

The acute toxicity test for turmeric extract follows the Food and Drug Administration of the Republic of Indonesia's guidelines, applying the OECD 420 Fixed-Dose method and administering stratified doses of turmeric extract of 300, 2000, and 5000 mg/kg. Twenty female Wistar rats were randomly assigned to two groups: the normal group (received the vehicle) and a group that received the turmeric extract. The extract given to the test animals in the treatment group started with the smallest dose (300 mg/kg). Additionally, the test animals were examined for 4 hours for signs of toxicity, followed by 24 hours of observation. Clinical signs were monitored every 30 minutes for the first four hours and then every hour for the next 24 hours. According to the guidelines, if no dead animals are observed following the low dose treatment, the amount then escalated to 5000 mg/kg.

2.6. Measurement of AST and ALT serum levels

According to the manufacturer's instructions, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using the Proline reagent kit. The absorbance of the test samples was determined using a Microlab 300 spectrophotometer at a wavelength of 340 nm.

2.7. Measurement of creatinine and urea levels

The serum creatinine and urea concentrations were determined using the Proline reagent kit following the manufacturer's instructions. At a wavelength of 340 nm, the absorbance of the test samples was determined using a Microlab 300 spectrophotometer.

2.8. Data analysis

The collected data were analyzed using the SPSS program version 18.0. The gathered data is presented in the form of mean values and standard deviations. There was a difference in the treatment group to the control group that was statistically significant at $p<0.05$.

3. Results

The positive group that received an L-NAME dose of 40 mg/kg for 21 days showed a significant increase in mean arterial pressure (MAP) compared to the normal group ($P = 0.02$) with a percentage of 66% (Table 1). The *Curcuma longa* extract at 50, 100, and 200 mg/kg doses significantly reduced blood pressure compared to the positive group. The effect of turmeric extract on decreasing blood pressure was comparable to that of the normal group and captopril dosed at 2.5 mg/kg in the group receiving turmeric extract (Table 1).

The group receiving turmeric fractions in n-hexane, ethyl acetate, and ethanol significantly reduced MAP compared to the positive group ($p<0.05$). Ethyl acetate fraction showed a more substantial effect on lowering blood pressure than other fractions (Table 2).

Acute toxicity testing with dosages of 300, 2000, and up to 5000 mg/kg revealed no evidence of experimental animal mortality (Table 3). Thus, the lethal dose of turmeric extract exceeds the 5000 mg/kg threshold for practical non-toxicity.

The findings of the Shapiro-Wilk data normality test for body weight on days 0, 7, and 14 indicated that they were normally distributed ($p>0.05$), and the homogeneity test resulted in uniformly distributed data ($p>0.05$). The independent T-test was used to continue the data analysis, which revealed a statistically significant difference ($p<0.05$) on days 7 and 14, where the group receiving turmeric extract observed an increase in body weight (Table 4).

Based on observations of clinical signs after the acute turmeric extract administration at a dose of 5000 mg/kg showed no clinical symptoms of toxicity. The observations for the next 14 days also showed no signs of toxicity (Table 5).

The Shapiro-Wilk data normality test revealed that the relative organ index of the heart and kidneys were normally distributed ($p>0.05$). The study was then repeated using an independent T-test, which revealed no statistically significant difference ($p>0.05$). The organ index result showed that the liver, lung, and spleen had statistically significant differences. ($p<0.05$). After conducting additional statistical analysis with the Kruskal-Wallis test, it was found that there was no statistically significant difference between the normal group and the turmeric extract dose of 5000 mg/kg ($p>0.05$). It demonstrates that 5000 mg/kg turmeric extract does not affect the organs index, such as the liver, kidneys, lungs, heart, or spleen (Table 6).

Following biochemical examination, the Shapiro-Wilk test demonstrated a normal data distribution for AST, ALT, creatinine, and urea ($p>0.05$). Further analysis using an independent T-test revealed no significant difference ($p>0.05$). The turmeric extract group showed significantly higher ALT and creatinine levels than the normal group ($p<0.05$). However, the increase was still within the normal range of organ function. Following statistical analysis, the Kruskal-Wallis test revealed no statistically significant variation between the groups ($p>0.05$). It demonstrates that acute turmeric extract ingestion at a dose of 5000 mg/kg has no harmful effect on liver or kidney function (Table 7).

Table 1. Results of mean arterial pressure (MAP) measurement results during extract treatment

Group	MAP (mmHg)		
	Day 0	Day 21	Day 42
Normal	79.75±12.99	79.75±8.80	75.75±10.37[a]
Positive	76.50±13.96	127±27.55[*]	125.75±19.98[*]
Captopril 2.5 mg/kg	75.50±3.31	128.75±12.97[*]	81.50±13.69[a]
Turmeric Extract 50 mg/kg	79.25±2.26	142.25±19.70[*]	90.25±15.26[a]
Turmeric Extract 100 mg/kg	81.75±3.40	134.75±18.11[*]	78.25±8.77[a]
Turmeric Extract 200 mg/kg	74.75±6.60	126.5±20.35[*]	66.75±8.05[a]

The values are expressed as mean±SD, n=5,
[*] There is a significant difference compared to the normal group ($p<0.05$),
[a] There is a considerable difference compared to the positive group ($p<0.05$)

Table 2. Results of mean arterial pressure (MAP) during the fraction treatment (n-hexane, ethyl acetate, and ethanol)

Treatment group	Mean MAP (mmHg)		
	Day 0	Day 21	Day 42
Normal	81.06±1.72	83.33±1.13	87.13±1.64[a]
Positive	80.09±0.78	109.99±3.01[*]	130.13±1.50
Captopril 2,5 mg/kg	82.33±1.10	119.66±3.45 [*]	95.19±1.67[a]
N-hexane fraction 25 mg/kg	82.39±0.82	118.13±3.28[*]	108.26±1.62[a]
Ethyl acetate fraction 25 mg/kg	81.86±2.11	122.59±3.29[*]	99.73±0.59[a]
Ethanol fraction 25 mg/kg	81.53±2.08	123.46±2.99[*]	105.46±1.64[a]

The values are expressed as mean±SD, n=5,
[*] There is a significant difference compared to the normal group ($p<0.05$),
[a] There is a considerable difference compared to the positive group ($p<0.05$)

Table 3. Percentage of deaths in test animals after administration of test drugs

Dosage (mg/kg)	Number of deaths in test animals	Percentage of deaths (%)
300	0	0
2000	0	0
5000	0	0

Table 4. Summary of body weight measurement in acute toxicity study of turmeric extract

Days	Bodyweight (mean±SD)	
	Control Group	Turmeric extract (5000 mg/kg)
Day 0	138.20±17.99	143.40±22.05
Day 7	141.70±19.11	163.50±16.38*
Day 14	144.20±19.40	162.30±17.07*

The values are expressed as mean±SD, n=10,
*There was a significant difference ($p<0.05$) compared to the control group.

Table 5. Summary of observations for behavioural signs after acute administration of turmeric extract

Toxic symptoms	The first 4 hours	24 hours	Day 3	Day 7	Day 10	Day 14
Autonomic nerve changes: exophthalmos, piloerection	N	N	N	N	N	N
Behaviour: depression, spinning	N	N	N	N	N	N
Sensory response	N	N	N	N	N	N
Muscle nerves: spasms	N	N	N	N	N	N
Blood vessels: bleeding	N	N	N	N	N	N
Breathing: breathless	N	N	N	N	N	N
Ocular: mydriasis and lacrimation	N	N	N	N	N	N
Gastrointestinal: diarrhea, bloody stool	N	N	N	N	N	N
Skin: alopecia, edema	N	N	N	N	N	N

Description N: normal

Table 6. The weight of the organs in proportion to the bodyweight following an acute dose of 5000 mg/kg turmeric extract

Organs	Organ index (Mean±SD)	
	Normal Group	*Turmeric extract* (5000 mg/kg) group
Heart	0.37±0.08	0.36±0.08
Liver	3.81±1.06	3.89±0.66
Lungs	1.10±0.33	1.04±0.33
Kidney	0.85±0.21	0.87±0.14
Spleen	0.69±0.16	0.58±0.17

The values are expressed as mean±SD, n=10, $p<0.05$

Table 7. A summary of biochemical analyses conducted during acute toxicity tests

Treatment groups	Parameter (mg/dl)			
	Creatinine	Urea	AST	ALT
Normal group	0.37±0.06	19.13±5.67	97.77±7.71	45.20±11.36
Turmeric extract 5000 mg/kg	0.43±0.11	22.10±4.15	93.77±10.25	50.57±7.39

The values are expressed as mean±SD, n=10, $p<0.05$, ALT: Alanine transferase, AST: Aspartate transferase

4. Discussion

It has been reported that administering L-NAME at a dose of 40 mg/kg for three weeks raises blood pressure, and cardiovascular remodeling is accompanied by decreased regulation of eNOS expression in the heart and aorta, resulting in a decrease in plasma NO levels (Bunbupha et al., 2015). The present study is in line with previous studies that tetrahydrocurcumin, the main bioactive compound of turmeric given at doses of 50 and 100 mg/kg, can reduce oxidative stress by lowering superoxide production and increasing glutathione. Furthermore, it increased O2 production and eNOS expression in arterial tissue in L-NAME-induced hypertensive rats. The underlying mechanism is due to the potent antioxidant properties of tetrahydrocurcumin, thereby increasing the bioavailability of nitric oxide (Sangartit et al., 2016). Other studies have also reported that tetrahydrocurcumin can improve vascular dysfunction, reduce blood pressure, and arterial stiffness in animal models of cadmium-induced hypertension. This effect occurs through antioxidant and anti-inflammatory activity (Sangartit et al., 2014). Curcumin has also been shown to improve hemodynamic function, endothelial dysfunction, vascular remodeling, and oxidative stress in hypertension animal models. It is associated with increased plasma nitrate/nitrite levels and expression of eNOS and a decrease in superoxide generation in vascular tissue (Boonla et al., 2014).

In vitro research studies reported that turmeric extract also inhibited an angiotensin-converting enzyme (ACE) activity, accompanied by increased NO production in L-NAME-induced hypertension (Akinyemi et al., 2015). It has been reported that treatment with curcumin can lower blood pressure in Angiotensin II (Ang II) induced hypertensive rats. Also, it is associated with inhibiting the Ang II type-1 receptor (AT1R) expression in the arteries, which exerts a vasoconstrictive effect mediated by Ang II. An increase in reactive oxygen species (ROS) can also increase the expression of AT1R in the kidneys (Yao et al., 2016).

In this study, the antihypertensive activity of turmeric fractions separated based on their polarity, all fractions showed a significant effect on reducing mean arterial pressure in test animals. This suggests that turmeric's chemical constituents, from polar to non-polar, can act synergistically to reduce blood pressure in L-NAME-induced test animals.

Animal groups that received turmeric extract at a dose of 5000 mg/kg showed an increase in body weight. It is likely due to the bioactive content of curcumin in turmeric extract, which can stimulate the gallbladder wall to release bile, thereby improving appetite (Prasad and Aggarwal, 2011). The bioactive plant substances are also known to improve digestion, which may lead to an increase in body weight (Toghyani et al., 2011). However, other research have demonstrated that the predominant quantity of curcumin from turmeric has an anti-obesity impact, suggesting that this action can suppress weight gain (Vafaeipour et al., 2022).

It is reported that curcuminoids, as compounds contained in turmeric, are essential substances in preventing kidney and liver diseases through their antioxidant effects (Sharma et al., 2011). Curcumin as an antioxidant can inhibit liver damage by binding to free radicals and is thought to increase apoptosis in liver damage so that this can be a protective mechanism (Zhong et al., 2016).

5. Conclusion

Turmeric rhizome extract has been shown to mitigate L-NAME-induced hypertension by reducing blood pressure and boosting blood flow throughout the body. The hypotensive impact was observed to be directly proportional to increasing the turmeric dose administered. The ethyl acetate fraction of turmeric was more hypotensive than the ethanol and n-hexane fractions. After acute administration of turmeric extract at a dose of 5000 mg/kg, no clinical symptoms of toxicity were seen. In conclusion, it can be stated that turmeric extract is entirely non-toxic and has no adverse effect on liver or kidney function.

Acknowledgement

The authors wish to express their gratitude to the Ministry of Research, Technology, and Higher Education (KEMENRISTEK DIKTI) for funding basic research through grant number 0001/P3M/E.PE/III/2019.

Conflict of interest

Authors declare no conflict of interest in this research.

References

Akinyemi AJ, Thome GR, Morsch V M, Stefanello N, Goularte JF, Belló-Klein A, Oboh G, Schetinger MRC. 2015. Effect of dietary supplementation of ginger and turmeric rhizomes on angiotensin-1 converting enzyme (ACE) and arginase activities in L-NAME induced hypertensive rats. *J Funct Foods* 17: 792–801. doi: 10.1016/j.jff.2015.06.011

Baradaran A, Nasri H, Rafieian-Kopaei M. 2014. Oxidative stress and hypertension: Possibility of hypertension therapy with antioxidants. *J Res Med Sci* 19(4): 358. PMID: 25097610; PMCID: PMC4115353.

Boonla O, Kukongviriyapan U, Pakdeechote P, Kukongviriyapan V, Pannangpetch P, Prachaney P, Greenwald SE. 2014. Curcumin improves endothelial dysfunction and vascular remodeling in 2K-1C hypertensive rats by raising nitric oxide availability and reducing oxidative stress. *Nitric Oxide* 42: 44–53. doi: 10.1016/j.niox.2014.09.001

Bunbupha S, Prachaney P, Kukongviriyapan U, Kukongviriyapan V, Welbat JU, Pakdeechote P. 2015. Asiatic acid alleviates cardiovascular remodelling in rats with L-NAME-induced hypertension. *Clin Exp Pharmacol Physiol* 42(11): 1189–97. doi: 10.1111/1440-1681.12472

Fleenor BS, Sindler AL, Marvi NK, Howell KL, Zigler ML, Yoshizawa M, Seals DR. 2013. Curcumin ameliorates arterial dysfunction and oxidative stress with aging. *Exp Gerontol* 48(2): 269–76. doi: 10.1016/j.exger.2012.10.008

Hasimun P, Mulyani Y, Sulaeman A, Embas Sara DA. 2019. Prevention of hypertension and arterial stiffness by combination of *Centella asiatica* and *Curcuma longa* in rats. *Asian J Biol Life Sci* 12(2): 173–9. doi: 10.3923/ajbs.2019.173.179

Inoue K, Nomura C, Ito S, Nagatsu A, Hino T, Oka H. 2008. Purification of curcumin, demethoxycurcumin, and bisdemethoxycurcumin by high-speed countercurrent chromatography. *J Agric Food Chem* 56(20): 9328–36. doi: 10.1021/jf801815n

Li R, Xiang C, Ye M, Li HF, Zhang X, Guo DA. 2011. Qualitative and quantitative analysis of curcuminoids in herbal medicines derived from *Curcuma* species. *Food Chem* 126(4): 1890–5. doi: 10.1016/j.foodchem.2010.12.014

Maneesai P, Bunbupha S, Kukongviriyapan U, Prachaney P, Tangsucharit P, Kukongviriyapan V, Pakdeechote P. 2016. Asiatic acid attenuates renin-angiotensin system activation and improves vascular function in high-carbohydrate, high-fat diet fed rats. *BMC Complement Altern Med* 16(1): 1–11. doi: 10.1186/s12906-016-1100-6

Meaney E, Alva F, Moguel R, Meaney A, Alva J, Webel R. 2000. Formula and nomogram for the sphygmomanometric calculation of the mean arterial pressure. *Heart* 84(1): 64. doi: 10.1136/heart.84.1.64

Mozaffarian D., Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ. 2015. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 131(4): e29–e322

Nakmareong S, Kukongviriyapan U, Pakdeechote P, Donpunha W, Kukongviriyapan V, Kongyingyoes B, Sompamit K, Phisalaphong C. 2011. Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with L-NAME-induced hypertension. *Naunyn-Schmiedeb Arch Pharmacol* 383(5): 519–29. doi: 10.1007/s00210-011-0624-z

Nakmareong S, Kukongviriyapan U, Pakdeechote P, Kukongviriyapan V, Kongyingyoes B, Donpunha W, Prachaney P, Phisalaphong C. 2012. Tetrahydrocurcumin alleviates hypertension, aortic stiffening and oxidative stress in rats with nitric oxide deficiency. *Hypertens Res* 35(4): 418–25. doi: https://doi.org/10.1038/hr.2011.180

Prasad S, Aggarwal BB. 2011. Turmeric, the golden spice. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd Ed.. Boca Raton: CRC Press/Taylor & Francis.

Sangartit W, Kukongviriyapan U, Donpunha W, Pakdeechote P, Kukongviriyapan V, Surawattanawan P, Greenwald SE. 2014. Tetrahydrocurcumin protects against cadmium-induced hypertension, raised arterial stiffness and vascular remodeling in mice. *PloS One* 9(12): e114908. doi: 10.1371/journal.pone.0114908

Sangartit W, Pakdeechote P, Kukongviriyapan V, Donpunha W, Shibahara S, Kukongviriyapan U. 2016. Tetrahydrocurcumin in combination with deferiprone attenuates hypertension, vascular dysfunction, baroreflex dysfunction, and oxidative stress in iron-overloaded mice. *Vascul Pharmacol* 87: 199–208. doi: 10.1016/j.vph.2016.10.001

Sharma V, Sharma C, Paliwal R, Pracheta SS, Sharma S. 2011. Ameliorative effects of Curcuma longa and curcumin on aflatoxin B1 induced serological and biochemical changes in kidney of male mice. *AJBPR* 1(2): 338–51

Sukandar EY, Permana H, Adnyana IK, Sigit JI, Ilyas RA, Hasimun P, Mardiyah D. 2010. Clinical study of turmeric (*Curcuma longa* L.) and garlic (*Allium sativum* L.) extracts as antihyperglycemic and antihyperlipidemic agent in type-2 diabetes-dyslipidemia patients. *Int J Pharmacol* 6(4): 456–63. doi: 10.3923/ijp.2010.456.463

Toghyani M, Toghyani M, Gheisari A, Ghalamkari G, Eghbalsaied S. 2011. Evaluation of cinnamon and garlic as antibiotic growth promoter substitutions on performance, immune responses, serum biochemical and haematological parameters in broiler chicks. *Livest Sci* 138(1-3), 167-73. doi: 10.1016/j.livsci.2010.12.018

Vafaeipour Z, Razavi BM, Hosseinzadeh H. 2022. Effects of turmeric (*Curcuma longa*) and its constituent (curcumin) on the metabolic syndrome: An updated review. *J Integr Med* 20(3), 193-203. doi: 10.1016/j.joim.2022.02.008

Yao Y, Wang W, Li M, Ren H, Chen C, Wang J, Wang WE, Yang J, Zeng C. 2016. Curcumin exerts its anti-hypertensive effect by down-regulating the AT1 receptor in vascular smooth muscle cells. *Sci Rep* 6(1): 1–8. doi: 10.1038/srep25579

Zhang Y, Lacolley P, Protogerou AD, Safar ME. 2020. Arterial stiffness in hypertension and function of large arteries. *Am J Hypertens* 33(4): 291–6. doi: 10.1093/ajh/hpz193

Zhong W, Qian K, Xiong J, Ma K, Wang A, Zou Y. 2016. Curcumin alleviates lipopolysaccharide induced sepsis and liver failure by suppression of oxidative stress-related inflammation via PI3K/AKT and NF-κB related signaling. *Biomed Pharmacother* 83: 302–13. doi: 10.1016/j.biopha.2016.06.036