Safety and /or Toxicity of Acute Dual Propolis and Ginger co- treatment in female albino Rats

Omayma AR. Abozaid [1], Omama E. El Shawi [2], Samy ,A.H..Aziza [1], Asmaa Abu-ElAzm [2]

[1]*Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Moshtoher, Benha, Egypt,*[2] *Department of health and Radiation Research, National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo Egypt.*

A B S T R A C T

The present study was conducted to evaluate the safety and / or the toxicity of acute dual propolis and ginger treatment. Different doses 1, 2, 3, 4 and 5 g/kg b.wt (w/w) of the dual composite were administrated as an aqueous solution via an oral gavage to female albino rats. No adverse effects or mortality were observed during the 72-hrs observation period. In addition, necropsy examination indicated no abnormalities in the main organs after administration of any dose. Further, histopathological, hematological indices (hemoglobin, red blood cells, hematocrite, and white blood cells) and plasma parameters included Alanine amino transferase (ALT) and aspartate amino transferase (AST) , total protein (TP) total cholesterol, triglycerides (TG), creatinine and urea, malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were assessed in the plasma of all rats after treatment with the limited oral dose 5 g/kg b.wt (w/w). There were no changes in histological architectures of brain and liver hematological and plasma chemistry values of the examined parameters. Collectively together, the hematological, biochemical and histological studies verified that oral dual administration of propolis and ginger in female rats was quite safe up to the limited dose used and did not cause any toxic symptoms or changes.

Key words: Propolis, Ginger, Dual oral treatment, safety and /or toxicity.

(http://www.bvmj.bu.edu.eg) **(BVMJ-34(1): 453-460, 2018)**

1. INTRODUCTION

Medicinal plants have increasingly become an integral part of the human society with regards to their therapeutic uses. Thus, phytomedicine research is now being promoted, as shown by the resolutions and recommendations given by the World Health Organization, which advocates the application of scientific criteria and methods for proof of safety and efficacy of medicinal plants (Ogbuehi et al., 2015).

Propolis is a resinous substance collected by *Apis mellifera* L. from buds and exudates of different plant sources. It is also mixed with beeswax, pollen, and some certain enzymes from bees' saliva (Pietta et al., 2002). The chemical composition of propolis is diverse and complex. Approximately, 300 compounds have been identified from propolis, including flavonoids, phenolic acids, terpenoids, steroids, and amino acids (Bankova et *al*., 2000). Propolis has been used as a traditional medicine for thousands of years; thus, it has been extensively investigated in many application fields (Tang et al., 2014). Propolis covers a broad spectrum of biological effects from anticancer (Sun et al., 2012) and antioxidant (Hatano et al., 2012) to antiviral (Vaijwade et al., 2014) and anti-inflammatory (Wang et al., 2014) properties. These biological properties can mainly be ascribed to phenolic compounds, in particular phenolic acids and flavonoids.

Ginger (*Zingiber officinale* Roscoe, Zingiberacae) is widely used in foods as a spice around the world and a traditional medicinal plant that has been widely used in Chinese, Ayurvedic and Unani-Tibb medicines for several thousand years (Ogbuehi et al., 2015). It contains large amount of phytochemicals such as flavonols, flavonone, anthocyanin, xanthin, flavonoids, tannins (Mukherjee et al., 2014).

Based on the above mentioned, both Propolis and ginger are known to be beneficial for human health and used as a folk medicine in many parts of the world. However, there was still lack of systemic safety evaluation. Important pharmacological properties like anti-oxidants, anti-bacterial, anti-fungal, anti-parasitic, anti-inflammatory, anti-cancer, immunomodulatory, cardioprotective, chemopreventive and radioprotective activities of ginger polyphenols have been reported (Bod and Dong, 2011).

Therefore, this work was designed -for the 1st time- to provide information about the safety and/ or systemic toxicity of oral acute co-administration of propolis and ginger in female albino rats.

2. MATERIALS AND METHODS

2.1. Ginger and Propolis

Ginger and Propolis: (100% Indian rhizome powder of the *Zingiber officinale*) and 100% natural dried propolis powder were Produced by Intiman Health Shop, the industrial area Obour City, Cairo, Egypt and purchased from (Emtenan) the local market (56 Mohamed Hassanieen Hykal, Abas Elakkad, Nasr City, Cairo, Egypt).

2.2. Chemicals and Kits

All chemicals used for measurement of lipid peroxidation marker antioxidants were purchased from Sigma Chemical Co. (St. Louis, MO). All kits used in biochemical analysis of the biochemical variables were supplied by Bio-diagnostic Company (Cairo-Egypt).

2.3. Experimental Animals

Female albino rats (110-120g) were obtained from the animal breeding house of National Centre for Radiation Research and technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

The animals were kept in isolated cages, under standard laboratory conditions including all hygienic measures with constant illumination and ventilation and normal conditions of temperature and humidity. Animals were maintained on a standard laboratory pellets containing all nutritive elements and free access to tap water was available. The animals were allowed to acclimatize for two weeks before the experiment.

All animal studies were conducted in accordance with criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals.

2.4. Animal treatment

This study was performed to verify the safety/toxicity of oral acute doses of aqueous suspension of propolis and ginger co administration in female albino rats.

- In this experiment, thirty (30) of female albino rats weighed (110 to 120 g) were divided randomly into six groups. The treated five groups rats co administered a single dose of subsequently (1, 2, 3, 4 and 5) g/kg /body wt. (wt/wt.) of the propolis/ginger suspended in distilled water (DW) by gastric gavage, while the control rats received only vehicle (DW).
- The animals received different doses of the dual treatment as presented in table 1.
- The animals were observed for 72h for any signs of acute toxicity and mortality in each group. The general behavior of the rats (changes in skin, hair, eyes, mucous membranes, and respiratory, circulatory, autonomic, and central nervous systems, abnormal behavior, motor activity, tremors, convulsion, salivation, diarrhea, lethargy, or sleep) was monitored continuously during the first 24h (,0.5,1,2,4, and 12hrs) and daily till the end of the observable period (72-hrs).
- The animals were fastened for 16-18h and sacrificed under light anesthesia using phenoparpital (30mg/kg). Then, they were rapidly dissected. All treated animals were

subjected for visual gross examination for any morphological changes in the internal visceral organs. The livers and the brains of the limited oral dose (5 g/kg) were excised and examined macroscopically.

The blood samples of the same group were collected on anticoagulant (heparin),devided into2 parts on part used for hematological analysis (Hemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC). The Second part was centrifugated for biochemical evaluation of Alanine amino transferase (ALT) and aspartate amino transferase (AST) (Reitmen and Frankel, 1957). Total protein (TP) (Doumas et al., 1971) total cholesterol (Allain et al., 1974), triacylglycerol (TG) (Fossati and Principe, 1982). Creatinine (Alan, 2006) and Urea (DI-Giorgio, 1974), Lipid peroxidation end product; malondialdehyde (MDA) (Ohkawa et al., 1979), Reduced glutathione (GSH) content (Beutler et al., 1963), superoxide dismutase (SOD)(Minami and Yoshikawa, 1979)and catalase (CAT) (Aebi, 1984) activities were also evaluated in the plasma of all rats.

2.5. Tissue processing for light microscopy

For light microscopic examination, pieces of the liver and brain tissue were fixed immediately after dissection in 10% formalin solution, embedded in paraffin wax, five µm-thick sections were cut and stained with hematoxylin/eosin according to the method of and examined under the light microscope and finally digital photos were recorded.

2.6. Statistical Analysis

The obtained data were expressed as mean ± standard error of the mean (M±SE). The significant differences among groups were determined by one-way analysis of variances (ANOVA) using SPSS package program, version 20. The results were considered significant if the values of p were <0.05.

3. RESULTS

3.1. Safety and /or toxicity evaluation of the dual administration of aqueous suspension of propolis and ginger in rats

Single oral doses of the aqueous suspension of propolis and ginger in combination were orally administered as presented in Table 1. Mortality, clinical signs were observed for 72 h after administration. In addition, necropsy was performed to examine any abnormalities in the main organs.

No appreciable changes in the general behavior of the rats (changes in skin, hair, eyes, mucous membranes, and respiratory, circulatory, autonomic, and central nervous systems, abnormal behavior, motor activity, tremors, convulsion, salivation, diarrhea, lethargy, or sleep) were noticed in different treated rats compared to the vehicle control.

Table 1: different doses of the dual treatment (Propolis and ginger) used for estimation of its safety and/ or systemic toxicity

Dose/g/Kg b. wt.	ml of the suspension /Kg		Ratio
	Propolis	Ginger	
1	5ml	5ml	
2	10ml	10ml	v:v
3	15ml	15ml	
4	20ml	20ml	
5	25ml	25ml	

3.2. Gross pathologic observations

Gross appearance of internal organs (liver, kidney, spleen, lung, heart ect.) of treated rats showed normal texture, shape, size and color compared to those of the vehicle control.

3.3. Histological Studies

Microscopically, Brain sections from treated rats, no notable histological changes were observed in brain sections of the treated rats which displayed normal structure the neuronal cells in the cerebral cortex (Fig. 1A), in the hippocampus (Fig. 1B) and in the Striatum(Fig. 1C) compared with vehicle control rats. Likewise, liver sections from the same group showed normal histological

architecture with normal hepatic lobule which consists of the central vein and the hepatocytes are concentrically around the central vein (fig 1-D).

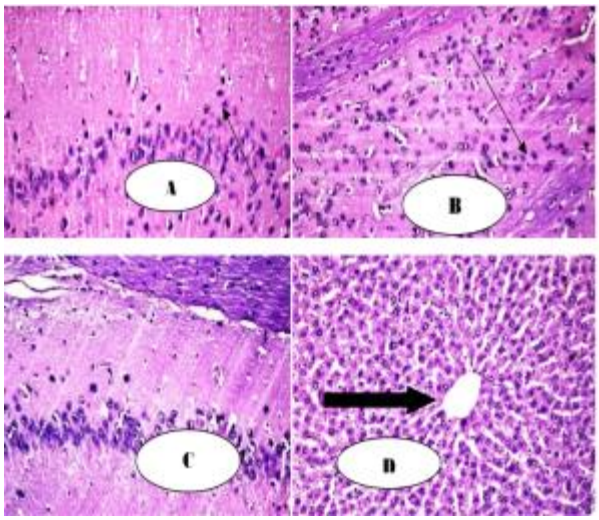

Figure 1: Sections in the brain and liver tissue of treated rats showing normal brain histological architecture of: A) the neuronal cells in the cerebral cortex, B) in the hippocampus and C) in the Striatum, D) normal histological structure of the hepatic vein (arrow) and the hepatocytes in parenchyma

3.4. Hematological analysis

The hematological indices were assessed after 72hrs of dual administration of propolis and ginger as a single oral dose of 5 g/kg (w/w). No changes in the examined hematological indices including hemoglobin (Hb), red blood cells (RBCs), Hematocrite (HCT) as well as white blood cells (WBCs) of the treated rats were related to the vehicle treatment (table 2).

Table 2: Hematological parameters in rats after the 72hours observation period following a single oral dose of dual propolis and ginger treatment5 g/kg of body weight (w/w)

Treatments	Parameters			
	Hb (g/dl)	RBCs (10^6/µl)	Ht (%)	WBCs (10^3/µl)
Vehicle control	14.51± 0.23	8.11± 0.07	45.30± 0.58	7.44± 0.62
Treated group	14.32± 0.18	8.20± 0.08	45.41± 0.53	7.79± 0.58

Data are expressed as mean ± SEM (n = 4 female rats) in each group. Differences between treatment groups and the control group were not significant (p>0.05)

3.5. Clinical Biochemistry analysis

Clinical chemistry parameters were assessed 72 hrs post of a single oral dose co administration of 5 g/kg b. wt (w/w) of propolis and ginger. No treatment related changes were noted in all measured parameters of treated rats compared to those of vehicle control (P≥0.05) (Tables 3, 4, and 5).

Table 3: Hepatic enzyme (ALT and AST) activities in plasma of rats after the 72 hours observation period following a single oral dose of propolis and ginger dual treatment at 5g/kg b.wt (w/w)

Treatments	Parameters in plasma	
	ALT (U/liter)	AST (U/liter)
Vehicle control	77.0 ± 2.6	11.3 ± 0.88
Treated group	78.03 ± 1.8	12.3±0.67

Data are expressed as mean ± SEM (n = 4 female rats) in each group. Differences between treatment groups and the control group were not significant

Table 4: hepatic metabolic activities in plasma of rats after the 72hours observation period following a single oral dose of propolis and ginger dual treatment at 5g/kg b.wt (w/w)

Treatments	Parameters		
	TP	Cholesterol	Triglycerides
Vehicle control	5.6± 0.2	47.6± 2.33	70.6± 1.2
Treated group	5.82± 0.24	43.3± 0.18	71.4± 1.06

Data are expressed as mean ± SEM (n = 4 female rats) in each group. Differences between treatment groups and the control group were not significant

Table 5: renal metabolites in plasma of rats after the 72hours observation period following a single oral dose of propolis and ginger dual treatment at 5g/kg b.wt (w/w)

Treatments	Parameters	
	Urea	Creatinine
Vehicle control	26.6 ± 7.7	0.08 ± 0.02
Treated group	25.88±6.07	0.083±0.01

Data are expressed as mean ± SEM (n = 4 female rats) in each group. Differences between treatment groups and the control group were not significant

Table 6: Oxidative marker (MDA) and antioxidant status (GSH, SOD and CAT) in plasma of rats after the 72 hours observation period following a single oral dose of propolis and ginger dual treatment at 5g/kg b.wt (w/w)

Treatments	Parameters			
	MDA (nmol/ml)	GSH (mg/ml)	SOD (U/ml)	CAT (U/ml)
Vehicle control	137.01± 2.33	7.2± 0.28	124± 1.51	68± 2.31
Treated group	131± 2.33*	9.3± 0.31**	144± 2.08**	77.1± 1.67*

Significant differences between treated and the control group at $^{*}p \leq 0.05$, $^{**}P \leq 0.01$. The aqueous oral suspension of propolis and ginger at a dose of 5g/kg caused consipeous brought down ($p<0.0$) in the MDA level, associated with significant elevation in GSH ($p<0.01$) content. SOD activity ($p<0.01$) and CAT ($p<0.05$) activity compared to the vehicle control rats

4. DISCUSSION

Various bioactive compounds in herbal plants possess the capacity to significantly modulate the complex mechanisms involved in the pathology of chronic diseases (Martinez-Augustin, et al., 2012). In the search for new sources of health-promoting constituents in herbal plants, in this study; we focused on the dual aqueous suspension of propolis and ginger to verify the safety and /or oral acute toxicity in female albino rats.

Our finding verified that oral co administration of aqueous suspension of Propolis and ginger at doses up to 5 g/kg b.wt. did not produce any demonstrable acute toxic effect or mortality in the treated rats. Accordingly, it could be concluded that oral LD50 of the suspension is higher than 5mg/kg b.wt. (W/w). Prior investigators have been evidenced that substances possessing LD50 higher than 50 mg/kg b.wt are non-toxic (Buck et al., 1976). Moreover, according to guidelines of the Organization for Economic Cooperation and Development (OECD, 2001), substances possessing LD50 dose of 2000 mg/kg b.wt. or higher are categorized as unclassified. So in agreement with that evidences, the tested dual suspension suggested being non-toxic when delivered orally up to 5g/kg (w/w).

Hematopoietic parameters are some of the most sensitive to assess the toxicity of drugs in humans and animals, and a blood profile usually gives vital information on the response of the body to injury or stress (Liju et al., 2013). Our findings demonstrated that, a single oral dose of the dual aqueous suspension (5g/kg) did not cause any significant changes in the measures hematological indices (table 2).

It has been emphasized that; the levels of Alanine transaminase (ALT) and aspartate transaminase (AST) which are indicative of liver function increased in liver tissue as well as plasma and considered as adaptive mechanism due to the stress of any drug (Sadeghi *et al*., 2016). Therefore, in our study, the possible hepatic toxicity could be induce by the dual aqueous suspension was demonstrated by evaluating the change in the levels of ALT and AST and TP, total cholesterol and TG as a sensitive biomarkers of liver activity (Tables 3, 4). Likewise there was no significant change in urea and creatinine (table 5) indicating no renal toxicity induced by the examined dual aqueous suspension.

These results could be explained on the basis that oral LD50 of Propolis extract in mice was between 2-7.3 g/kg b.wt.(Banskota et al., 2001). The authors concluded that the dose of 70mg/day was extrapolated as safe for human. In addition; (Mohammadzadeh et al., 2007) showed that oral administration of hydroalcoholic solution of Propolis extract in rats at doses of (4.5, 9, 13 and 20 g/kg b.wt) has no toxic effects. On the other hand, Rong and colleagues demonstrated that oral administration of ginger powder up to 2000 mg/kg to male and female rats was not associated with any mortalities and

abnormalities in general conditions, behavior, growth of male and female rats (Rong et al., 2009). On the other hand, Ginger is generally considered to be a safe herbal medicine with only a few, non-significant adverse side effects (Bae and Kim, 2011). A group of researchers reported that no treatment-related mortalities were observed after a single oral dose of steamed and dried ginger extract within 14 days of treatment up to 5,000 mg/kg (Kim and Choi, 2017). Acute toxicity of ginger was reported after administering an 80% ethanol extract in mice. At 2.5 g/kg, no mortality was observed, although two out of ten animals experienced mild diarrhea. However, doses of 3 and 3.5 g/kg resulted in moralities of 20 and 30%, respectively, within 72 h of administration. The acute oral dose at which 50% mortality was observed (LD50) in rats, and the acute dermal LD50 in rabbits of ginger oil, exceeded 5 g/kg body weight (Anonymous, 2003).

The oxidation and the antioxidant status was evaluated in plasma of treated rats compared to vehicle control rats and the results were recorded in table 6 indicating the decrement of MDA level coupled with increment of GSH content and SOD and CAT activities. These results could be attributed to the antioxidant and scavenging activities of propolis (Alam Eldein et al., 2017) and ginger (Mashhadi et al., 2013) due to the high content of polyphenolic composites such flavonoids, tannins and terpenoids (El-Naggar et al., 2015, Mukherjee et al., 2012). It is therefore, could function as preventive agents against oxidative damage (El-Naggar et al., 2016). Due to the antioxidants activity and the free radical scavenging properties of both propolis and ginger, the endogenous status of treated rats was enhanced as indicated by the elevation of GSH content, as well as SOD and CAT activities and consequently caused brought down of MDA level in the present study.

Conclusion: Overall, the experiment outcome suggests that oral co administration of aqueous suspension of propolis and ginger was quite safe up to the limited dose used in this study (5gm/kg w/w), thus forming a basis for further studies on the sub-acute and /or chronic doses of this pharmacologically important dual treatment.

5. REFERENCES

Aebi, H. (1984) Catalase in Vitro. Method Enzym.105: 121-126.

Alan, H. B (2006) Tietz Clinical Guide to Laboratory Tests 4th ed, W.B. Saunders Company. Elseiver

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W., Fu, P.C (1974) Enzymatic determination of total serum cholesterol. Clin Chem. 20(4):470-5.

Alm-Eldeen, A.A., Basyony, M.A., Elfiky, N.K., Ghalwash, M.M (2017) Effect of the Egyptian propolis on the hepatic antioxidant defense and pro-apoptotic p53 and anti-apoptotic bcl2 expressions in aflatoxin B1 treated male mice. Biomedicine & Pharmacotherapy 87,: 247–255.

Anonymous, A (2003) Zingiberis rhizome, In: ESCOP Monographs. Thieme Press Stuttgart, New York, pp. 547-553.

Bae, J.S., Kim, T.H (2011) Pancreatic lipase inhibitory and antioxidant activities of Zingiber officinale extracts. Korean J. Food Preserv.; 18: 390-396.

Bankova, V. S., De Castro, S. L. and Marcucci, M. C. (2000) "Propolis: recent advances in chemistry and plant origin," *Apidologie*, vol.31, no. 1, pp. 3–15, 2000.

Banskota, A.H., Tezuka, Y., Shigetoshi, K (2001) recent progress in pharmacological research of propolis. Phytother. Res. 15, 561–571.

Beutler, E., Duron, O., kelly, B.M (1963) Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61:882-8.

Bode, A.M., Dong, Z (2011) the amaizing and mighty ginger, Herbal Medicine: Biomolecular and Clinical Aspects. 2nd

ed. Boca Raton (FL): CRC Press/Taylor & Francis; Chapter 7.

Buck, W.B., Osweiter, G.D., Van Gelder, A.G (1976) Clinical and Diagnostic Veterinary Toxicology, second ed. Kendall/hunt Publishing Co., Iowa, p. 5211.

DI-Giorgio, J (1974) Nonprotein nitrogenous constituents, In : clinical chemistry – principles and technics, 2 nd ed. RJ Henry, DC Cannon, JW Winkelman, editors, Harper and Row, Hagerstown (MD).;41-553.

Doumas, B.T., Watson, W.A., Biggs, H.G (1971) Albumin standards and the measurement of serum albumin with bromcresol green Clin. Chim. Acta. Jan; 31(1):87-96.

Ellman, G. L., Courtney, D. K., Andres V. and Featherstone, R. M. (1961).“A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity,” Biochemical Pharmacology, Vol.7, No. 2, pp. 88-95.

El-Naggar, S.A., Abdel-Farid, I.B., Germoush, M.O., Elgebaly, H.A. and Alm- Eldeen, A.A. (2016). Efficacy of Rosmarinus officinalis leaves extract against cyclophosphamide-induced hepatotoxicity, Pharm. Biol.

El-Naggar, S.A., Alm-Eldeen, A.A., Germoush, M.O., El-Boray, K.F. and Elgebaly, H.A. (2015) Ameliorative effect of propolis against cyclophosphamide-induced toxicity in mice, Pharm. Biol. 53 235–241.

Fossati, P., Prencipe, L (1982) Serum triglycerides determined colorimetric-cally with an enzyme that produces hydrogen peroxide. Clin Chem. 28(10): 2077-80.

Ganesh, C. Jagetia, M., Shrinath B., Ponemone, V. and Jagadish N. U. (2003) Influence of Ginger Rhizome (Zingiber officinale Rosc) on Survival, Glutathione and Lipid Peroxidation in Mice after Whole-Body Exposure to Gamma Radiation Radiation Research; 2003 , 160 (5): 584- 592.

Hatano, A. Nonaka, T., Yoshino, M (2012) “Antioxidant activity and phenolic constituents of red propolis from Shandong, China,” Food Science and Technology Research, 18(4) 577–584.

Kim, J.Y. and Choi, J.S (2017) Single-dose oral toxicity and acute dermal irritation of steamed and dried ginger extract in rat and white rabbit. The Journal of Animal & Plant Sciences, 27(6) 1822-1828.

Liju, V.B., Jeena, K., Kuttan, R. (2013) Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (*Curcuma longa* L.). *Food Chem. Toxicol., 53*, 52–61.

Martinez-Augustin, O., Aguilera, C.M., Gil-Campos, M., Sanchez de Medina, F., Gil, A (2012) Bioactive anti-obesity food components. Int. J. Vitam. Nutr. Res., 82, 148–156.

Mashhadi, N.S., Ghiasvand, R., Askari, G., Hariri, M., Darvishi, L., and Mofid, M.R (2013) Anti-Oxidative and Anti-Inflammatory Effects of Ginger in Health and Physical Activity: Review of Current Evidence. Int J Prev Med.; 4(1): S36–S42.

Minami, M. and Yoshikawa, H. A (1979) simplified assay method of superoxide dismutase activity for clinical use. Clin Chim. Acta.; 92:337.

Minami, M., and Yoshikawa, H (1979) A simplified assay method of superoxide dismutase activity for clinical use. Clin Chim Acta. Mar 15;92(3):337-42

Mohammadzadeh, S., Shariatpanahi, M., Hamedi, M., Ahmadkhaniha, R., Samadi, N. and Ostad, S.N (2007) Chemical composition, oral toxicity and antimicrobial activity of Iranian propolis. Food Chemistry 103, 1097–1103.

Mukherjee, S., Mandal, N., Dey, A. and Mondal, B (2014) An approach towards optimization of the extraction of polyphenolic antioxidants from ginger (Zingiber officinale). J Food Sci Technol; 51(11):3301–3308.

OECD (Organization for Economic Cooperation and Development) (2001) OECD guideline for testing of chemicals at <http://www.oecd.org>

Ogbuehi, I. H., O., Ebong, O., Atuboyedia W.O (2015) Oral acute toxicity (LD50)

study of different solvent extracts of Abrus precatorius Linn leaves in wistar rats. European Journal of Experimental Biology, , 5(1):18-25.

Ohkawa, H., Ohishi,N. and Yagi, K.(1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal.Biochem.*1979; 95: 351-358.

Pietta, P. G. Gardana, C.and Pietta A. M.,(2002) "Analytical methods for quality control of propolis," *Fitoterapia*, vol. 73, no. 1, pp. S7–S20, 2002.

Reitmen, S., Frankel S. (1957) Colorimetric test for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path.; 25:56.

Ronga, X., Gang, P., Takuya, S., Qinglin, Y., Johji, Y., Yuhao, L. A (2017) 35-day gavage safety assessment of ginger in rats. The Journal of Animal & Plant Sciences, 27(6) 1822-1828.

Sadeghi, L. A., Farzeen, T., Vahid, Y. B. (2016) Antioxidant effects of alfalfa can improve iron oxide nanoparticle damage: Invivo and invitro studies. Regulatory Toxicology and Pharmacology 81

Sun, L.P. Chen, A. L., Hung H. C (2012) "Chrysin: a histone deacetylase 8 inhibitor with anticancer activity and a suitable candidate for the standardization of chinese propolis," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 47, pp. 11748–11758, 2012.

Tang, T.-X., Guo W.-Y., Xu Y (2014) "Thin-layer chromatographic identification of Chinese propolis using chemometric fingerprinting," Phytochemical Analysis, 25(3): 266–272.

Vaijwade, D. N. S. Kulkarni, S. R. and Sanghai, N. N. (2014)."Screening of antiviral compounds from plants—a review," *Journal of Pharmacy Research*, vol. 8, no. 8, pp. 1050–1058, 2014.

Wang, K. Zhang, J. L. Ping S.(2014). "Anti-inflammatory effects of ethanol extracts of Chinese propolis and buds from poplar (*Populus×canadensis*)," *Journal of Ethnopharmacology*, vol. 155, no. 1, pp. 300–311.

Young, D.S (2001) Effects of Disease on Clinical Laboratory Tests.4 ed. Washington, DC: American Association for Clinical Chemistry.