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Review

# Tremendous health benefits and clinical aspects of *Smilax china*

Mohamad Hesam Shahrajabian<sup>1,2</sup>, Wenli Sun<sup>1,2</sup> and Qi Cheng<sup>1,2\*</sup>

<sup>1</sup>Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China.

<sup>2</sup>Nitrogen Fixation Laboratory, Qi Institute, Building C4, No. 555 Chuangye Road, Jiaxing 314000, Zhejiang, China.

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A lot of species of *Smilax* known as Baqia in China are used in folk medicine for various purposes. *Smilax china* L., is a small vine that grows in the southern parts of China, known as Jin Gang Ten, which has a long history of indigenous use in China. *S. china* consists of fat, saponins, glucosides, gum, starch, flavonoids, tannins, and alkaloids. *S. china* has been used in traditional Chinese medicine because it has effective components such as triterpenoid, saponins, flavones, stilbenes, and organic acids. Roots are the most common used part; stems and rhizome can be used also in the form of powder or paste, raw or cooked. The most important health benefits of *S. china* are energy tonic, impotency and seminal disorders, chronic arthritis and secondary and tertiary syphilis, schizophrenia and epilepsy, pemphigus and skin diseases, osteo-arthritis, leucorrhea or white discharge, relieving joints numbness, diabetes and excretory system. The obtained findings strongly suggest potential of *S. china* as an additive in pharmaceutical industries.

**Key words:** Health benefits, *Smilax china*, pharmaceutical industries, traditional Chinese medicine.

## INTRODUCTION

The use of traditional Chinese herbs and fruits for the treatment and management of diseases is common in developing countries and it is improving in developed countries (Soleymani and Shahrajabian, 2012; Ge et al., 2018; Shahrajabian et al., 2018; Shahrajabian et al., 2019a,b,c,d). In recent years, pharmacokinetic and metabolic studies of traditional Chinese medicine have attracted extensive attention and promoted in many regions (Ogbaji et al., 2018; Soleymani and Shahrajabian, 2018). The genus *Smilax* (Liliaceae family) comprises about 300 species of climbing flowering shrub (Xie et al., 2018). Some of the *Smilax* plant distributed in Asia area includes Taiwan, China, and Japan (Huang,

2000). China cultivates this drug in large amount; hence, it is usually recognized as China root. The most important popular common names of the plants are China root, Chinese smilax and Bambook Briar Root. Many species of *Smilax* are known as Baqia in China and are used in folk medicine for various purposes (Ao, 2013). Shu et al. (2006) reported that *Smilax china* L., is a small vine that grows in the southern parts of China, known as Jin Gang Ten, which has a long history of indigenous use in China. Yang et al. (2008) found that the rhizome of *S. china* has been used in traditional Chinese medicine because it has effective components such as triterpenoid saponins, flavones, stilbenes and organic acids. Local

\*Corresponding author. E-mail: [chengqi@caas.cn](mailto:chengqi@caas.cn).

**Table 1.** Local names of *Smilax china* L. in different languages.

Latin name	Smilax china
English name	China root
Hindi name	Chopchini, Chobchini, Toupchini
Mizoram name	Kaitha
Tamil name	Ayadi
Malayalam name	Kaltamara
Marathi name	Ghotvel
Telugu name	Kondadantena
Kannada name	Kaaduhambu
Bengali name	Kumarika
Oriya name	Mootrilata

**Table 2.** Phytochemical tests of *Smilax china* (Saravanakumar et al., 2014).

Group test	Name of the test	Observation
Carbohydrate and gums	Molish test	+
Reducing sugar	Fehling's Solution test	-
	Benedict's test	-
Alkaloids	Mayer's test	-
	Dragendorff's test	-
	Wagner's test	-
	Hagner's test	+
Steroides	Salkowski reaction	+
	Libermann-burchared reaction	+
Glycosides	Salkowski reaction	+
	Libermann-burchared reaction	-
Tannins	Ferric chloride test	-
	Potassium dichromate test	+
	Keller-Kiliani test	+
Flavonoids	Hydrochloric acid test	-
Saponins	Foam test	-

names of *S. china* L. in different languages are shown in Table 1.

### CHEMICAL CONSTITUTIONS

*S. china* consists of fat, saponins, glucosides, gum, starch, flavonoids, tannins and alkaloids (Saravanakumar et al., 2014). Feng et al. (2003) showed that 5 phenyl compounds were isolated from the roots of *S. china* and they are dihydrokaempferol (1), 3,5,4'-trihydroxystilbene

(2), 3,5,2',4'-tetrahydroxystilbene (3), dihydrokaempferol 3-O- $\alpha$ -L-rhamnoside (engeletin, 4), and quercetin 4'-O- $\beta$ -D-glucoside (5). Shao et al. (2009) found that seven flavonoids and four stilbenes were isolated and identified as dihydrokaempferol-5-O- $\beta$ -D-glucoside (I), engeletin (II), isoengeletin (III), dihydroquercetin-3-O-glycoside (IV), 3, 5, 7, 3', 5'-pentahydroxy-flavanonol (V), astilbin (VI), quercetin-3'-O-glycoside (VII), piceid (VIII), scirpusin A (IX), resveratrol (X), and oxyresveratrol (XI). Results of phytochemical tests of *S. china* are shown in Table 2. Names of flavones and isoflavones isolated from *S. china*

**Table 3.** The names of flavones and isoflavones which isolated from *S. china* L. (Zhao et al., 2016).

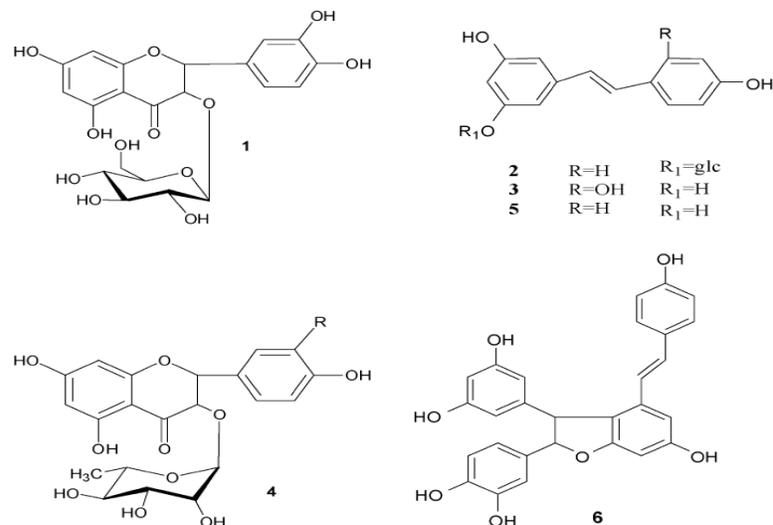
Flavones	Kaempferol
	Kaempferide
	Morin
	Kaempferol 7-O- $\alpha$ -L-ranmnoside
	Kaemperin
	Quercetin-4'-O- $\beta$ -D-glucoside
	Vitexin
	Kaempferitrin
	Lepidoside
Rutin	
Isoflavones	Pratensein
	Puerarin
	Smilachinin

L. are shown in Table 3. Shao et al. (2007) reported that the six major active constituents in *S. china* are (1) Taxifolin-3-O-glycoside; (2) piceid; (3) oxyresveratrol; (4) engeletin; (5) resveratrol; (6) scirpusin A. Structural compounds 1 to 6 identified from *S. china* are as shown in Figure 1.

## HEALTH BENEFITS

*S. china* L. known as Jin Gang Ten, has been widely used as a traditional herbal medicine for the treatment of gout, rheumatoid arthritis and other diseases for a long time in China (Chen et al., 2011). Shu et al. (2004) confirmed that the tuber of *S. china* L. has anti-inflammatory, anticancer, and anticoagulation activities. In Chinese medicine, it has been extensively used for clinical treatment of syphilis, acute bacillary dysentery acute, chronic nephritis and antitumor (Chen et al., 2002). The rhizomes of *S. china* is commonly used as herbal materials in traditional Chinese medicine (Liang et al., 2016). Park et al. (2014) concluded that *S. china* methanol extract (SCME) has active compounds which have anti-obesity activities. Vijayalakshmi et al. (2013) reported that the ethyl acetate fraction of *S. china* rhizome showed maximum antipsoriatic activity. Chen et al. (2011) concluded that *S. china* L. exhibits anti-hyperuricemic and nephroprotective activity in hyperuricemic animals. Jeong et al. (2013) reported that *S. china* has antimicrobial, antimutagenic, antioxidant, anti-inflammatory, anti-cancer and neuroprotective effects. Shim (2012) also recognized *S. china* has a good source of natural antioxidant. Raju et al. (2012) also showed that *S. china* is an anti-diabetic plant which is responsible for the hypoglycemic activities. Bhati et al. (2011) reported that the hydroalcoholic and aqueous fractions exhibited anti-diabetic activity in rats with alloxan-induced diabetes. Seo et al. (2012) indicated that *S. china* L. possesses antioxidant and antimicrobial substances,

and suggested that the ethanol extract can be applied into food and cosmetic industry. Wu et al. (2010) showed that polyphenols are the active components of *S. china* L. responsible for the anti-breast tumor cell activities. Saraswathi and Nithya (2010) suggested that the hypoglycemic and hypolipidemic property of *S. china* could be useful for the treatment of diabetes. Sarvana and Felicia (2015) also claimed that *S. china* extracts have antioxidant activity which can be used to treat various diseases. Shu et al. (2006) stated that ethyl acetate extract of *S. china* possesses remarkable anti-inflammatory effects on acute inflammation, and also displays anti-inflammatory effects on the chronic inflammation at a certain extent. Pan et al. (2014) concluded that water extraction from *S. china* (WESC) suppressed fat accumulation and decreased the weight gain in mice, which was mainly due to increase of the activity of fat oxidation enzyme in liver, promotion of the fatty acid  $\beta$ -oxidation. Lee et al. (2016) suggested that the extract from *S. china* L. has great potential as a cosmetic ingredient with whitening effects. Vijayalakshmi et al. (2012) have found the flavonoid quercetin in *S. china* and they have stated that it is promising for further investigations to prove its anti-psoriatic activity. Cong et al. (2016) noted that those patients who received Azithromycin therapy added with *S. china* capsules concurrently could significantly improve levels of lymphocyte subsets, cytokines and hemorheology index. Yang et al. (2019) stated that *S. china* L. ethanol extract (SCLE) could lead to a decrease in body weight gain and fat mass by inhibiting the lipid synthesis and promoting lipolysis and  $\beta$ -oxidation in high-fat diet (HFD) fed mice. Pharmacological studies have also suggested that *S. china* has a neuroprotective effect (Ban et al., 2006). Lee et al. (2018) demonstrated the potent therapeutic efficacy of *S. china* L., and its potential use as a cost-effective natural alternative medicine against type 2 diabetes and its complications. Nho et al. (2015) reported that *S. china*



**Figure 1.** Structural compounds 1-6 identified from *S. china*. (1) Taxifolin-3-O-glycoside; (2) piceid; (3) oxyresveratrol; (4) engeletin; (5) resveratrol; (6) scirpusin A (Shao et al., 2007).

**Table 4.** The most important traditional uses and benefits of China root.

Root is alternative, anti-scrophulatic, carminative, depurative, diaphoretic, diuretic and tonic.

It is useful when taken internally in the treatment of old syphilitic cases and it also used for certain skin diseases, including psoriasis, rheumatoid, arthritis, gout, enteritis, urinary tract infections, skin ulcers, etc.

Large doses can cause nausea and vomiting, which is appreciated in weakened and depraved conditions due to a poisoned state of the blood.

*Smilax* is helpful in improving muscle mass and body strength.

It is used as a tonic for male sexual energy.

*Smilax* has a special property as it acts against the problems caused due to malnourishment of Dhatus such as poor immunity and weakness.

Decoction of roots and rhizomes used as depurative in cases of herpeticism and syphilis.

It is Sudorific and demulcent, used in rheumatism.

It is used for various skin diseases.

It is used as a depurative, diaphoretic, stimulant, alterative, antisiphilitic and asphrodisiac

It is used as alterative in old syphilitic cases and in chronic rheumatism.

In TCM, used as diuretic and for treatment of rheumatic arthritic conditions; also used for detoxification, treatment of gout, tumors and lumbago.

It is used for syphilis, skin disease, epilepsy, insanity, flatulence, dyspepsia, constipation, fever, neuralgia, rheumatism, gout and general debility in Ayurveda, Siddha and Unani medical system.

It is used as a remedy for inflammatory disease and ischuria.

Rhizome is made into a paste and applied to painful swellings.

It has also been supported in the treatment of leprosy, scrofula and many skin infections developing into ulcers.

Roots have been used to treat abscesses, pyoderma and burns.

It was one of the drugs used in the treatment of acute appendicitis, taeniasis and constipation.

Roots have been used to treat cases of paralysis and sciatica.

It is used to treat urinary tract infection, stone and ulcers of the bladder and even chyluria by the physicians.

It is also used to treat fever and other inflammatory conditions associated with fever like acute lymphadenitis.

It helps in relieving strangury and also seminal weakness.

*L. extract* (SCLE) exerts an anti-metastatic effect on human breast cancer cells. The most important traditional

uses and benefits of *S. china* are shown in Table 4. The most important health benefits of China root are shown in

**Table 5.** The most important health benefits of China root.

Energy tonic
Impotency and seminal disorders.
Chronic arthritis and secondary and tertiary syphilis.
Schizophrenia and epilepsy.
Pemphigus and skin diseases.
Osteo-arthritis
Leucorrhoea or white discharge.
Relieving joint numbness
Diabetes
Excretory system.

Table 5.

## CONCLUSION

*S. china* L. known as China root has been used for thousand years in numerous tribal and folk medicine. The plant is native to China, Korea, Taiwan, Japan, Philippines, Vietnam, Thailand, Myanmar and Assam. *S. china* consists of fat, saponins, glucosides, gum, starch, flavonoids, tannins and alkaloids. The rhizomes are bitter, acrid, thermogenic, anodyne, anti-inflammatory, digestive, laxative, depurative, diuretic, febrifuge and tonic. It is used in dyspepsia, flatulence, colic, constipation, helminthiasis, skin diseases, leprosy and psoriasis, syphilis, strangury, seminal weakness, general debility, detoxifies organs, cleanses blood, aids absorption and kills bacteria; it is also used for fever, epilepsy, insanity, neuralgia and stimulates digestion, increases urination, protects liver and promotes perspiration. In Chinese medicinal science, it has been used for clinical treatment of syphilis, acute bacillary dysentery acute, chronic nephritis and antitumor. On the basis of scientific literatures, *S. china* L. demonstrates important and promising health benefits. In general, treatment with natural and traditional medicine, especially *S. china* L is recommended.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Ao CQ (2013). Anther wall development, placentation, sporogenesis and gametogenesis in *Smilax davidiana* A.DC: A contribution to the embryology of *Smilax*. South African Journal of Botany 88:459-465.
- Ban JY, Cho SO, Koh SB, Song KS, Bae K (2006). Protection of amyloid  $\beta$  protein (25-35)-induced neurotoxicity by methanol extract of *Smilax china* rhizome in cultures rat cortical neurons. Journal of Ethnopharmacology 106:230-237.
- Bhati R, Singh A, Saharan VA, Ram V, Bhandari A (2011). Pharmacognostical standardization, extraction and anti-diabetic activity of *Smilax china* L. rhizome. Asian Journal of Traditional Medicines 6(5).
- Chen L, Yin H, Lan Z, Ma S, Zhang C, Yang Z, Li P, Lin B (2011). Anti-hyperuricemic and nephroprotective effects of *Smilax China* L. Journal of Ethnopharmacology 135(2):399-405.
- Chen T, Li JX, Cai Y, Xu Q (2002). A flavonol glycoside from *Smilax glabra*. Chinese Chemical Letters 13:537-538.
- Cong RJ, Jiang XH, Zhang H (2016). Effect of Smilax China capsules and azithromycin combined therapy on chronic annexitis. Journal of Hainan Medical University 22(22):120-123.
- Feng F, Liu WY, Chen YS, Liu JH, Zhao SX (2003). Flavonoids and stilbenes from *Smilax China*. Journal of China Pharmaceutical University 34(2):119-121.
- Ge J, Hu Y, Guo L, Wang C, Sun W, Shahrajabian MH (2018). Effects of Gas and ABA on the germination of dormant oat seeds. Cercetari Agronomice in Moldova 3(175):25-41.
- Huang TC (2000). Flora of Taiwan. Department of Botany, National Taiwan University, Taipei. 5:103-106.
- Jeong CH, Jeong HR, Kwak JH, Kim JH, Choi GN, Kim DO, Lee U, Heo HJ (2013). Phenolic composition and in vitro antioxidant activities of *Smilax China* root. Journal of Food Biochemistry 37(1).
- Lee HE, Kim JA, Whang WK (2018). Chemical constituents of *Smilax china* L. stems and their inhibitory activities against glycation, aldose reductase,  $\alpha$ -glucosidase, and lipase. Molecules 22:451.
- Lee SY, Yoo DH, Joo DH, Jo HS, Lee JY (2016). Inhibitory efficacy of smilax China L. on MITF, TRP-1, TRP-2, tyrosinase protein and mRNA expression in melanoma cell (B16F10). Korean Journal of Microbiology and Biotechnology 44(1):1-8.
- Liang F, He JW, Zhu GH, Liu RH, Shu JC, Shao F, Huang HL (2016). New steroidal saponins with cytotoxic activities from *Smilax trinervula*. Phytochemistry Letters 16:294-298.
- Nho KJ, Chun JM, Kim HK (2015). Anti-metastatic effect of *Smilax china* L. extract on MDA-MB-231 cells. Molecular Medicine Reports 11:499-502.
- Ogbaji PO, Li J, Xue X, Shahrajabian MH, Egrinya EA (2018). Impact of bio-fertilizer or nutrient solution on Spinach (*Spinacea Oleraceae*) growth and yield in some province soils of P.R. China. Cercetari Agronomice in Moldova 2(174):43-52.
- Pan YF, Zhang GD, Zhang QF, Li DM (2014). Effects of water extract Smilax china on body weight and fat metabolism of high-fat diet induced obese mice. Modern Food Science and Technology 30(2):12-16.
- Park JA, Jin KS, Kwon HJ, Kim BW (2014). The anti-obesity effect of *Smilax china* extract. Korean Journal of Microbiology and Biotechnology 42(4):354-360.
- Raju BGS, Rao BG, Manju YB (2012). Antidiabetic activity of Smilax china roots in alloxan-induced diabetic rats. International Journal of PharmTech Research 4(1).
- Saraswathi U, Nithya NR (2010). Antihyperglycemic and antihyperlipidemic property of *Smilax china* in alloxan induced diabetic rats. Asian Journal of Microbiology, Biotechnology and Environmental Sciences 12(1):49-53.
- Saravanakumar S, Felicia C, Subramanian S (2014). Pytochemical screening of the methanol extract of root tuber Smilax China.

- International Journal of Pharmacognosy and Phytochemical Research 6(4):963-966.
- Seo HK, Lee JH, Kim HS, Lee CK, Lee SC (2012). Antioxidant and antimicrobial activities of *Smilax china* L. leaf. Food Science and Biotechnology 21(6).
- Shahrajabian MH, Khoshkharam M, Sun W, Cheng Q (2019a). Germination and seedlings growth of corn (*Zea mays* L.) to allelopathic effects of rice (*Oryza sativa* L.). Tropical Plant Research 6(1):152-156.
- Shahrajabian MH, Khoshkharam M, Zandi P, Sun W, Cheng Q (2019b). Jujube, a super-fruit in traditional Chinese medicine, heading for modern pharmacological science. Journal of Medicinal Plants Studies 7(4):173-178.
- Shahrajabian MH, Sun W, Cheng Q (2019c). Clinical aspects and health benefits of ginger (*Zingiberofficinale*) in both traditional Chinese medicine and modern industry. Acta Agriculturae Scandinavica, Section B: Soil and Plant Science, pp. 1-11.
- Shahrajabian MH, Sun W, Cheng Q (2019d). Modern pharmacological actions of longan fruits and their usages in traditional herbal remedies. Journal of Medicinal Plants Studies 7(4):179-185.
- Shahrajabian MH, Wenli S, Cheng Q (2018). A review of goji berry (*Lyciumbarbarum*) in traditional Chinese medicine as a promising organic superfood and superfruit in modern industry. Academia Journal of Medicinal Plants 6(12):437-445.
- Shao B, Guo HZ, Cui YJ, Liu AH, Yu HL, Guo H, Xu M, Guo DA (2007). Simultaneous determination of six major stilbenes and flavonoids in *Smilax china* by high performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis 44:737-742.
- Shao B, Guo HZ, Guo DA (2009). Flavonoids and stilbenes from *Smilax China*. Chinese Traditional and Herbal Drugs 40(11):1700-1703.
- Shim SM (2012). Changes in profiling phenolic compounds, antioxidative effect and total phenolic content in *Smilax china* under invitro physiological condition. Journal of Food Biochemistry 36(6).
- Shu XS, Gao ZH, Yang XL (2006). Anti-inflammatory and anti-nociceptive activities of *Smilax china* L. aqueous extract. Journal of Ethnopharmacology 103:327-332.
- Shu XS, Gao ZH, Yang XL (2006). The anti-inflammation effects of *Smilax china*ethylacetate extract in rats and mice. ZhongguoZhongyaozazhi (Zhongguozhongyaozazhi = China Journal of Chinese Material Medical) 31(3):239-243.
- Shu XS, Gao ZH, Yang XL (2004). Supercritical fluid extraction of sapogenins from tubers of *Smilax china*. Fitoterapia 75:656-661.
- Soleymani A, Shahrajabian MH (2012). Response of different cultivars of fennel (*Foeniculum vulgare*) to irrigation and planting dates in Isfahan, Iran. Research on Crops 13(2):656-660.
- Soleymani A, Shahrajabian MH (2018). Changes in germination and seedling growth of different cultivars of cumin to drought stress. Cercetari Agronomice in Moldova 1(173):91-100.
- Vijayalakshmi A, Ravichandiran V, Malarkodi V, Sankaradoss N, Anusha Male, Jayakumari S, Ravichandiran V (2013). Anti-psoriatic activity of *Smilax china* Linn. Rhizome. Indian Journal of Pharmaceutical Education 47(1):82-89.
- Vijayalakshmi A, Ravichandiran V, MalarkodiVelraj, Nirmala S, Jayakumari S (2012). Screening of flavonoid quercetin from the rhizome of *Smilax china* Linn. For anti-psoriatic activity. Asian Pacific Journal of Tropical Biomedicine 2(4):269-275.
- Wu LS, Wang XJ, Wang H, Yang HW, Jia AQ, Ding Q (2010). Cytotoxic polyphenols against breast tumor cell in *Smilax china* L. Journal of Ethnopharmacology 130(3).
- Xie Y, Hu D, Zhong C, Liu KF, Fang E, Zhang YJ, Zhou C, Tian LW (2018). Anti-inflammatory furostanolsaponins from the rhizomes of *Smilax china* L. Steroids 140:70-76.
- Yang C, Tang Q, Liu J, Zhang Z, Liu W (2008). Preparative isolation and purification of phenolic acids from *Smilax china* by high-speed counter-current chromatography. Separation and Purification Technology 61:474-478.
- Yang L, Zhao Y, Pan Y, Li D, Zheng G (2019). Dietary supplement of *Smilax china* L. ethanol extract alleviates the lipid accumulation by activating AMPK pathways in high-fat diet fed mice. Nutrition and Metabolism 16:6.
- Zhao BT, Le DD, Nguyen PH, Ali MdY, Choi JS, Min BS, Shin HM, Rhee HI, Woo MH (2016). PTP1B,  $\alpha$ -glucosidase, and DPP-IV inhibitory effects for chromene derivatives from the leaves of *Smilax china* L. Chemico-Biological Interactions 253:27-37.

*Full Length Research Paper*

## Effect of the anticancer drug tamoxifen on chronic toxoplasmosis in experimentally infected rats

Rabab Fawzy Selem<sup>1,2</sup>, Gehan Abdel Rahman Rashed<sup>1</sup>, Ashraf Mohamed Abdel Khalek Barakat<sup>3</sup>, Hasan Mohamed Ali Elfadaly<sup>3</sup>, Boshra EL-sayed Talha Hussien<sup>4</sup>, Hemat Salah Mohamed<sup>1</sup>, Marwa Mohamed Nageeb<sup>1</sup> and Ahlam Farag Moharm<sup>1\*</sup>

<sup>1</sup>Parasitology Department, Faculty of Medicine, Benha University, Egypt.

<sup>2</sup>Taif Faculty of Medicine, Kingdom of Saudia Arabia.

<sup>3</sup>Department of Zoonotic Diseases, Veterinary Research Division, National Research Center, Egypt.

<sup>4</sup>Tropical Medicine Department, Faculty of Medicine, Tanta University, Egypt.

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***Toxoplasma gondii* is an opportunistic parasite that can cause severe disorders in infants and pregnant women and can also be lethal in immunologically compromised individuals. During unfit host immune conditions, and as a consequence to latent stage opportunity, the protozoan stimulates serious infection, and signifies higher morbidity and mortality including humans with Acquired Immuno-Deficiency Syndrome (AIDS) or those receiving corticosteroids and cancer chemotherapy. Tamoxifen drug (TAM) is a selective estrogen receptor modulator (SERM), which is commonly used for treatment of breast cancer; it has a known immunomodulatory effects on the patient, especially if administered for a long time as happens in cases of post breast cancer surgery and anti-recurrence prophylactic measures where women might persist to take TAM for years. The research question here was: Can TAM reactivates latent toxoplasmosis? To assess the possible stressful effect of TAM, rats were experimentally infected by *T. gondii* (RH strain). Three months later, they were treated by oral administration of TAM (10 mg/kg body weight/day) for 7, 14, 21 and 28 days. Tamoxifen effect on toxoplasmosis dynamics was estimated by counting *Toxoplasma* brain cysts and serological detection of anti-parasitic IgM and IgG all through the experiment time. The results showed an initial insignificant decrease in parasitic burden in groups treated for one week followed by a significant increase in groups treated for 14, 21 and 28 days. There was also a significant decrease in IgM titers in groups treated for one and two weeks while there was a significant increase in IgM titers in groups treated for three and four weeks. There was a significant increase in IgG titers in groups treated for 14 and 21 days and a border line significant increase in the 1<sup>st</sup> week while there was non-significant increase in 4<sup>th</sup> week.**

**Key words:** Toxoplasmosis, tamoxifen, breast cancer, serological, *Toxoplasma* brain cysts, immunomodulatory.

### INTRODUCTION

*Toxoplasma gondii* (*T. gondii*) is the causal agent of toxoplasmosis and one-third of the world population had

\*Corresponding author. E-mail: drahlammoharm@yahoo.com. Tel: 00201098053493.

been affected by this parasite (El-On and Peiser, 2003). In immune suppressed individuals, such as those undergoing chemotherapy, organ transplantation or in AIDS patients, reactivation of a latent *T. gondii* infection is often fatal. *T. gondii* has been identified as an important opportunistic infection in HIV/AIDS patients and a major contributor to death of AIDS patients in the developing world (Carruthers and Suzuki, 2007). Host affection was confirmed a sequel to reactivation of primary infection (Saadatnia and Golkar, 2012). Breast cancer is the most common malignancy in women around the world. Information on the incidence and mortality due to breast cancer is essential for planning preventive health measures (Ghoncheh et al., 2016). Treatment with TAM lowers the risk of breast cancer recurrence and also lowers the risk of death from breast cancer (Early Breast Cancer Trialists' Collaborative Group, 2011). Since both toxoplasmosis and breast cancer are widely distributed globally, the research question was: Could tamoxifen treatment lead to reactivation of latent primary toxoplasmosis? For that purpose, we treated toxoplasmosis experimentally infected rats with TAM for different periods and observed the possible stressful effects of it on *Toxoplasma* parasitosis.

## MATERIALS AND METHODS

### Study site

This study was conducted in National Research Center; NRC (Cairo, Giza).

### Ethical considerations

The study was approved by the Parasitology Department Research Committee and the Ethical Committee at the Faculty of Medicine, Benha University.

### Parasites

*Toxoplasma gondii* (RH strain) was obtained from Zoonotic Diseases Department, National Research Center, Egypt. Tachyzoites of *T. gondii* (RH) strain maintained through serial intra-peritoneal (i.p.) passage were used for experimental infection. Tachyzoites were collected from mouse peritoneal cavity 72 h post infection (p.i.), the parasites were counted and adjusted to  $10^3$ /ml in saline. Each 1 ml solution was inoculated subcutaneously into each experimental rat

### Drugs

Tamoxifen (nolvadex) (Sigma-Aldrich) 10 mg tablets was orally administered to the rats at a dose of 10 mg/Kg body weight daily (Perumal et al., 2005), via oral gavage 90 days post infection for 1, 2, 3 and 4 weeks. Tablets were dissolved in sunflower oil (Sigma-Aldrich) and the dose was adjusted for each rat according to its weight.

### Animals, infection and treatment schedule

To test the efficacy of tamoxifen in a chronic model of experimental

toxoplasmosis, a total of 45 laboratory-bred male rats were used (10 weeks old, weighing ~250 g). Animals were housed and maintained in a suitable rearing environment with free access to food and water throughout the experiment. Infected rats were divided into four groups consisting of 10 to 11 rats each group (7 infected and treated + 3-4 rats served as positive control; infected non treated) in addition to 3 healthy non infected- non treated; negative control rats. The first group was treated by tamoxifen for 7 days, the second group treated for 14 days, the third one treated for 21 and fourth group treated for 28 days. At the end of each group treatment time, rats were sacrificed, their brains were dissected and examined for immediate direct parasitological assessment and blood samples were collected individually, sera were separated and kept at -20°C for later serological evaluation.

## Evaluation of tamoxifen efficacy

### Parasitological assessment

To prepare the brain suspension, rats were sacrificed, brains were removed and prepared in a tissue homogenizer (Wheaton USA) with 1 ml saline each. For cyst enumeration, 0.1 ml of the brain suspension was placed on a slide. The number of *Toxoplasma* cysts was counted in ten high power fields (HPF) and then the mean number was determined for each rat followed by calculation of the mean numbers of cysts in each infected group (Djakovic and Milenkovic, 2001).

### Serological assay

Serum samples were serologically assayed by ELISA to detect IgM & IgG titer according to procedures described by Lind et al. (1997).

### Statistical analysis

Gathered data were tabulated and analyzed using SPSS statistical software (IBM Corp., Armonk, NY, USA). Data were expressed as mean  $\pm$ SD. Analysis of variance between groups was done using t test. P value < 0.05 was considered statistically significant.

## RESULTS

The results showed that there was a decrease in average brain parasitic load (ABPL) in TAM infected and treated (IT) group for one week as compared to the infected untreated (IU) control group (3.4%), however the difference was statistically non-significant ( $p = 0.448$ ). Inversely, in the other IT groups which were treated for 14, 21 and 28 days, there were a statistically significant ( $p = 0.0001 - 0.0045$ ) increases in ABPL (by 11.4, 30.3 and 48.7%, respectively) as shown in Table 1. In the serological study, there was a statistically significant decrease ( $p = 0.0001 - 0.027$ ) in Anti *Toxoplasma* IgM titers in IT groups treated for 7 and 14 days, inversely, in IT groups treated for 21 and 28 days, a statistically significant increase ( $p = 0.0001 - 0.0012$ ) was found in anti-*Toxoplasma* IgM titers as shown in Table 2. Concerning anti *Toxoplasma* IgG titers, throughout the

**Table 1.** Average brain parasite load (ABPL) of Tamoxifen treated rats as compared with untreated rat at different time points.

DPI / DPT	Group/average brain parasite load (ABPL/10 mg/brain)			p value	T
	(I U) BPL	(I T)BPL	AGD (NO.) (%)		
90 (IPL)	26553.67±1031.253	-			
97(7DPT)	24206±1024.24	23364.86±1665.88	-841.14/ ↓3.4%	0.448	0.79
104(14DPT)	21141.25±1151.39	23861.71±1159.16	+2720.46 ↑ 11.4 %	0.0045*	3.75
111(21DPT)	19836.75±1239.87	28443.14±1843.32	8606.39 ↑30.2%	0.0001*	8.23
118(28DPT)	16335.67±875.22	31922.71±1884.4	+15587.05 ↑ 48.7%	0.0001*	13.36

ABPL: Average brain parasite load; IPL: Initial parasite load; IU: Infected untreated; IT: Infected treated; DPT: Day post treatment; DPI: Day post infection; AGD: Average difference between treated and untreated groups.

Experiment duration, the antibody generally increased starting from the first week and continued increasing till the fourth week, however those rises in IgG titers were statistically significant ( $p = 0.0062 - 0.0315$ ) in groups treated for 14 and 21 days, borderline significant ( $p = 0.0579$ ) in animals treated for 1 week and insignificant ( $p = 0.188$ ) in groups treated for 28 days as shown in Table 3.

## DISCUSSION

*T. gondii* is an obligate intracellular, parasitic protozoan. It is the etiologic agent for toxoplasmosis. About 30 to 50% of the world population is infected with the parasite, and it is the most prevalent infection among humans (Tenter et al., 2000; Flegr et al., 2014). Cluster of differentiation (CD4<sup>+</sup>) and (CD8<sup>+</sup>) T cells are highly activated during infection and are essential for adaptive immunity. As such, patients with defects in T cell-mediated immune responses (for example, patients with AIDS) are at risk for reactivation of latent *T. gondii* infections. CD4<sup>+</sup> and CD8<sup>+</sup> T cells act synergistically to prevent cyst reactivation during chronic latent *T. gondii* infection. CD8<sup>+</sup> T cells mediate protection against toxoplasmosis primarily through the generation of interferon- gamma (IFN- $\gamma$ ). Interleukin-12(IL-12) drives the generation of terminally differentiated CD8<sup>+</sup> effector T cells (Aliberti, 2005). CD4<sup>+</sup> T cells are critical for avoiding reactivation of latent toxoplasmosis, as the emergence of severe toxoplasmosis is concomitant with the decline in T cell numbers in patients infected with HIV (Luft et al., 1984; Israelski and Remington, 1988) and in mouse models, the lack of CD4<sup>+</sup> T cells is associated with increased susceptibility of reactivation during the chronic stage of infection (Johnson and Sayles, 2002). CD8<sup>+</sup> T cell

responses to *T. gondii* are influenced by good functioning provided by CD4<sup>+</sup> T cells (Lutjen et al., 2006). CD4<sup>+</sup> T cells are necessary for the maintenance of CD8<sup>+</sup> T cell effector functions during the chronic stage of infection, and this help must be provided during the acute stage of infection (Lutjen et al., 2006). Tamoxifen (TAM) is a broadly known anti-estrogen, which has been used in adjuvant treatment of early stage, estrogen-sensitive breast cancer for over 20 years, especially for women who still have significant ovarian estrogenic activity which could not be controlled by aromatase inhibitors. Five years of adjuvant tamoxifen safely reduced 15-year risks of breast cancer recurrence and death (Behjati and Frank, 2009; Early Breast Cancer Trialists' Collaborative Group, 2011). It has also immunomodulatory effects. Tamoxifen is capable of inducing a shift from cellular (T-helper 1) to humoral (T-helper 2) immunity. Interestingly, the immune modulatory effects of tamoxifen appear to be independent of the estrogen-receptor and may be mediated through the multi-drug resistance gene product (Behjati and Frank, 2009). Robinson et al. (1993) studied the effects of tamoxifen on immunity in patients with bilateral breast cancer who were in remission and had completed radiotherapy and chemotherapy at least one year prior to the study. They observed that the relative proportion and absolute number of CD4<sup>+</sup> lymphocytes was reduced in tamoxifen treated patients, compared to untreated breast cancer patients and to healthy controls. Moreover, *in vitro* proliferation of lymphocytes derived from tamoxifen treated patients was decreased. Since CD4<sup>+</sup> cells have the main role in immunity against *T. gondii* whether on their own or by promotion of CD8<sup>+</sup> cells as mentioned above, so theoretically, letting down CD4<sup>+</sup> cells number or activity - as recorded before for TAM - can impair host immunity against toxoplasmosis. A community where both

**Table 2.** Optical density (ODs) of anti-toxoplasma IgM ELISA titers in Tamoxifen treated rats as compared with control groups.

Result	Group									
	Uninfected control ODs	Infected control ODs	1 week ODs		2 weeks ODs		3 weeks ODs		4 weeks ODs	
		90 DPI	97 DPI	7 DPT	104 DPI	14 DPT	111DPI	21 DPT	118DPI	28 DPT
	0.179	0.678	0.623	0.535	0.524	0.363	0.346	0.416	0.246	0.638
	0.151	0.708	0.564	0.496	0.502	0.389	0.279	0.375	0.313	0.626
	0.209	0.734	0.617	0.521	0.539	0.417	0.352	0.513	0.258	0.574
				0.611	0.521	0.354	0.293	0.439		0.583
				0.515		0.403		0.507		0.562
				0.489		0.342		0.488		0.546
				0.541		0.406		0.528		0.713
<b>Mean OD</b>	0.179±0.029	0.706±0.028	0.601±0.032	0.529±0.04	0.521±0.015	0.382±0.028	0.317±0.036	0.466±0.057	0.272±0.035	0.606±0.057
<i>P</i> -value between uninfected control and other groups	-	0.0001* t=22.63	0.0001* t=16.77	0.0001* t = 13.36	0.0001* t=20.53	0.0001* t=10.11	0.003* t=5.31	0.0001* t=8.037	0.025 t = 3.48	0.0001* t=11.87
<i>P</i> -value between each treated and untreated groups at the same time point			0.027* t=2.68		0.0001* t=8.82		0.0012 t= 4.62		0.0001* t=9.11	

toxoplasmosis and breast cancer are commonly recorded was the targeted one by this research, the assumption here was that TAM can lead to reactivation of latent toxoplasmosis through its immune modulatory effect mentioned above, consequently lead to unrecognizable exacerbated *T. parasitosis* with its possible serious sequelae on patients who are already devastated by the primary oncogenic condition. In this study, parasitologically, it was observed that the average brain parasitic load (ABPL) in infected treated (IT) rat group after one week decreased but the difference was statistically non-significant (3.4%). Inversely, there was a statistically significant

increase in (ABPL) in other (IT) rat groups treated for 14, 21 and 28 days by 11.4, 30.3 and 48.7.1%, respectively. In the same line were the results of serological study of anti-*Toxoplasma* IgM antibodies; there was a significant decrease in Anti *Toxoplasma* IgM optical density in (IT) rat groups treated for 7 and 14 days followed by a significant increase in (IT) rat groups treated for 21 and 28 days. Assessing Anti *Toxoplasma* IgG antibodies showed that they generally increased throughout the experimental period with no initial decrease as happened with ABPL & IgM titers, which may be explained by the fact that IgG antibodies persist for a longer period after the

primary infection in the infected host than IgM, so the general rise of IgG titers as compared to IgM may be attributed to the persisting IgG antibodies with the primary infection plus those generated as a result of infection reactivation. The initial decrease in both ABPL and anti-*Toxoplasma* IgM titers could be explained by the lethal effect of TAM on *Toxoplasma* parasites observed earlier by Dittmar et al. (2016) who explained that by the fact that estrogen was previously shown to increase the numbers of *Toxoplasma* tissue cysts in the brains of parasite-infected mice. Since TAM is the best-characterized antiestrogen inhibitor, it has anti *Toxoplasma* effects (Pung and Luster,

**Table 3.** Optical density (ODs) of Anti *Toxoplasma* IgG ELISA titers in Tamoxifen treated rats as compared with control groups.

Result	Group									
	Uninfected control ODs	Infected control ODs	1 week ODs		2 weeks ODs		3 weeks ODs		4 weeks ODs	
			97 DPI	7 DPT	104 DPI	14 DPT	111 DPI	21 DPT	118 DPI	28 DPT
	0.227	1.254	1.306	1.331	1.514	1.487	1.577	1.612	1.703	1.647
	0.154	1.312	1.342	1.396	1.472	1.582	1.624	1.718	1.682	1.749
	0.186	1.276	1.351	1.378	1.456	1.616	1.587	1.631	1.674	1.725
				1.365	1.383	1.553	1.492	1.655		1.684
				1.414		1.644		1.587		1.745
				1.362		1.639		1.646		1.808
				<b>1.363</b>		<b>1.547</b>		<b>1.711</b>		1.783
<b>Mean OD</b>	0.189± 0.036	1.280± 0.029	1.333±0.0238	1.372± 0.0267	1.456± 0.054	1.581± 0.056	1.570± 0.055	1.651± 0.048	1.686± 0.014	1.734±0.055
<i>P</i> -value between uninfected control and other groups	-	0.0001* t=40.34	0.0001* t=45.38	0.0001* t=58.15	0.0001* t=34.4	0.0001* t=38.45	0.0001* t=36.88	0.0001* t=46.21	0.0001* t=65.59	0.0001* t=43.65
<i>P</i> -value between each treated and untreated groups at the same time point			<b>0.0579?</b> <b>t = 2.211</b>		<b>0.0062*</b> <b>t= 3.55</b>		<b>0.0315*</b> <b>t=2.52</b>		<b>0.188</b> <b>t = 1.437</b>	

1986).Dittmar et al.(2016) also indicated that TAM inhibited *Toxoplasma* replication via a mechanism independent of its ability to antagonize estrogen receptor (ER) signaling even though they found that *Toxoplasma* activates ER-dependent transcription. In addition, they showed that tamoxifen reduced the overall number of parasite vacuoles and also induced the accumulation of LC3-green fluorescent protein (GFP) on the parasitophorous vacuole membrane (PVM). These data point to a mechanism by which tamoxifen kills *Toxoplasma* by inducing xenophagy. Xenophagy is now a well-recognized mechanism used by IFN-γ and CD40<sup>+</sup> to control *Toxoplasma* replication (Choi et al., 2014;

Andrade et al., 2006). However, with continuing TAM administration to rats for another two weeks, it was observed that ABPL, IgM and IgG titers were vividly increased denoting that TAM induced a concrete reactivation of latent toxoplasmosis in subjected rats as proven comparing their ABPLs, IgM, IgG titers with those belonging to the control groups. It was assumed that with continuation of TAM treatment, its immune modulatory effects mediated through shifting from TH1 to TH2 cells and decreasing CD4 numbers as reported previously (Rotstein et al., 1988; Robinson et al., 1993; Behjati1 and Frank, 2009), contradicted and predominated its anti toxoplasmic effects reported before by Dittmar et al. (2016) yielding a flare up

of infection as estimated by both parasitological and serological parameters.This study concluded that TAM treatment in chronically infected mice with *T. gondii* protozoon parasite resulted in initial control of infection then flare up and exacerbation of infection.Thus more studies was recommended on wider scale and for longer periods for assessment of the cost/benefit and medical rationale of screening patients of breast cancer on adjuvant TAM treatment for *Toxoplasma* infection before the start of and during the course of treatment so as to detect early any incoming reactivation of chronic infection by observation of the rising titers of anti-*Toxoplasma* IgM and IgG to guard against the fatal risk and complication of

toxoplasmosis in such immunocompromised patients.

There was a significant decrease in anti-*Toxoplasma* IgG optical density in (IT) group treated for 7, 14 and 21 days, and there was rising in anti-*Toxoplasma* IgG optical density in groups treated for 28 days but with no significant difference between (IU) and (IT) groups. These results confirmed the previous findings of ABPL and anti-toxoplasma IgM titre (Dittmar et al., 2016). The initial decrease of ABPL, IgM and IgG titers, might be due to the anti-*Toxoplasma* effects of tamoxifen reported previously by Dittmar et al. (2016) who explained that by the fact that estrogen was previously shown to increase numbers of *Toxoplasma* tissue cysts in the brains of parasite-infected mice. Since TAM is the best-characterized antiestrogen inhibitor, it has anti-*Toxoplasma* effects (Pung and Luster, 1986). Dittmar et al. (2016) also indicated that TAM inhibited *Toxoplasma* replication via a mechanism independent of its ability to antagonize estrogen receptor (ER) signaling even though they found that *Toxoplasma* activates ER-dependent transcription. In addition, they showed that tamoxifen reduced the overall number of parasite vacuoles and also induced the accumulation of LC3-green fluorescent protein (GFP) on the parasitophorous vacuole membrane (PVM). Together, these data point to a mechanism by which tamoxifen kills *Toxoplasma* by inducing xenophagy. Xenophagy is now a well-recognized mechanism used by IFN- $\gamma$  and CD40<sup>+</sup> to control *Toxoplasma* replication (Choi et al., 2014; Andrade et al., 2006). However, with continuing TAM administration to rats for another two weeks, it was observed that ABPL, IgM and IgG titers were vividly increased denoting that TAM induced a concrete reactivation of latent toxoplasmosis in subjected rats as proven by comparing their ABPLs, IgM and IgG titers with those belonging to the control groups. It was assumed that with continuation of TAM treatment, its immune modulatory effects mediated through shifting from TH1 to TH2 cells and decreasing CD4<sup>+</sup> numbers as reported before (Rotstein et al., 1988; Robinson et al., 1993; Behjati and Frank, 2009), contradicted and predominated its anti toxoplasmic effects yielding a flare up of infection as estimated by both parasitological and serological parameters. This study concluded that TAM treatment in chronically infected mice with *T. gondii* protozoan parasite resulted in initial control of infection then flare up and exacerbation of infection. Thus it was recommended that screening of *Toxoplasma* infection in patients of breast cancer on adjuvant TAM treatment before the start of and during the course of treatment so as to early detect any incoming reactivation of chronic infection by rising titer of anti-*Toxoplasma* IgM and IgG to guard against the fatal risk and complication of toxoplasmosis in such immunocompromised patients. More studies on TAM and toxoplasmosis in humans should be done.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Aliberti J (2005). Host persistence: exploration of anti-inflammatory pathways by *Toxoplasma gondii*. *Nature Reviews Immunology* 5(6):512.
- Andrade RM, Wessendarp M, Gubbels MJ, Striepen B, Subauste CS (2006). CD40 induces macrophage anti-*Toxoplasma gondii* activity by triggering autophagy-dependent fusion of pathogen-containing vacuoles and lysosomes. *Journal of Clinical Investigation* 116:2366-2377.
- Behjati S, Frank MH (2009). The Effects of Tamoxifen on Immunity. *Current Medicinal Chemistry* 16(24):3076-3080.
- Carruthers VB, Suzuki Y (2007). Effects of *Toxoplasma gondii* infection on the brain. *Schizophrenia Bulletin* 33(3):745-751.
- Choi J, Park S, Biering SB, Selleck E, Liu CY, Zhang X, Fujita N, Saitoh T, Akira S, Yoshimori T, Sibley LD, Hwang S, Virgin HW (2014). The parasitophorous vacuole membrane of *Toxoplasma gondii* is targeted for disruption by ubiquitin-like conjugation systems of autophagy. *Immunity* 40:924-935.
- Dittmar AJ, Drozda AA, Blader J (2016). Drug repurposing screening identifies novel compounds that effectively inhibit *Toxoplasma gondii* growth. *mSphere* 1(2):42-15.
- Djakovic D, Milenkovic T (2001). Murine model of drug-induced reactivation of *Toxoplasma gondii*. *ActaProtozool* 40:99-106.
- Early Breast Cancer Trialists' Collaborative Group (2011). Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *The Lancet* 378(9793):771-784.
- El-On J, Peiser J (2003). *Toxoplasma* and toxoplasmosis. *Harefuah* 142:48-55.
- Flegel J, Prandota J, Sovièková M, Israili ZH (2014). Toxoplasmosis—a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. *Public Library of Science PLoS One* 9(3):e90203.
- Ghoncheh M, Pournamdar Z, Salehiniya H (2016). Incidence and Mortality and Epidemiology of Breast Cancer in the World. *Asian Pacific Journal of Cancer Prevention* 17(3):43-46.
- Israelski DM, Remington JS (1988). Toxoplasmic encephalitis in patients with AIDS. *Infectious Disease Clinics of North America* 2(2):429-445.
- Johnson LL, Sayles PC (2002). Deficient humoral responses underlie susceptibility to *Toxoplasma gondii* in CD4-deficient mice. *Infection and Immunity* 70(1):185-191.
- Lind P, Haugegaard J, Wingstrand A, Henriksen SA (1997). The time course of the specific antibody response by various ELISAs in pigs experimentally infected with *Toxoplasma gondii*. *Veterinary Parasitology* 71(1):1-15.
- Luft BJ, Brooks RG, Conley FK, McCabe RE, Remington JS (1984). Toxoplasmic encephalitis in patients with acquired immune deficiency syndrome. *Journal of the American Medical Association* 252(7):913-917.
- Lutjen S, Soltek S, Virna S, Deckert M, Schluter D (2006). Organ- and disease-stage-specific regulation of *Toxoplasma gondii*-specific CD8-T-cell responses by CD4 T cells. *Infection and Immunity* 74(10):5790-5801
- Perumal SS, Shanthi P, Sachdanandam P (2005). Augmented efficacy of tamoxifen in rat breast tumorigenesis when gavaged along with riboflavin, niacin, and CoQ10: effects on lipid peroxidation and antioxidants in mitochondria. *Chemico-biological interactions* 152(1):49-58.
- Pung OJ, Luster MI (1986). *Toxoplasma gondii*: decreased resistance to infection in mice due to estrogen. *Experimental Parasitology* 61:48-56.
- Robinson E, Rubin D, Mekori T, Segal R, Pollack S (1993). In vivo

- modulation of natural killer cell activity by tamoxifen in patients with bilateral primary breast cancer. *Cancer Immunology Immunotherapy* (37):209-212.
- Rotstein S, Blomgren H, Petrini B, Wasserman J, Von Stedingk LV(1988). Influence of adjuvant tamoxifen on blood lymphocytes. *Breast Cancer Research and Treatment* 12:75-79.
- Saadatnia G, Golkar M (2012).A review on human toxoplasmosis. *Scandinavian Journal of Infectious Diseases* 44(11):805-814.
- Tenter AM, Heckeroth AR, Weiss LM (2000) .*Toxoplasma gondii*: from animals to humans. *International Journal for Parasitology* 30:1217-1258.

*Full Length Research Paper*

# Prevalence and indication of gabapentin and pregabalin prescriptions among adults in King Abdulaziz Hospital in Makkah AL-Mukarramah, KSA

Salma Alotaibi<sup>1</sup>, Arwa Alotaibi<sup>1</sup>, Ashwaq Almqati<sup>1</sup>, Khloud Alotaibi,<sup>1</sup> Atyaf Bogar<sup>1</sup> and Yosra Alhindi<sup>2</sup>

<sup>1</sup>Pharm D Intern students, Faculty of pharmacy, University of Umm Al-Qura, Makkah, KSA.

<sup>2</sup>Assistant Professor in clinical pharmacy, Faculty of pharmacy, University of Umm Al-Qura, Makkah, KSA.

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**Gabapentin and pregabalin prescribing have increased substantially over the recent years. Some evidence supports that gabapentin and pregabalin use in non-neuropathic pain disorders indicates they are less effective than several other licensed non-opioid analgesics. On the other hand, other studies have shown that those drugs are to be beneficial in the treatment of non-neuropathic pain and improves the analgesic efficacy of opioids both at rest and in movement, reduces analgesic consumption and opioid-related adverse effects. Therefore, it is essential to evaluate the rate of their prescriptions as well as monitoring and checking any severe side effects. The study is aimed at identifying the rate and the indications of gabapentin and pregabalin prescriptions at King Abdul-Aziz (Alzaher) Hospital-Makkah. A cross-sectional study was conducted from medical records of in-patients and outpatients clinics from January, 2018 through January, 2019. Data analysis was performed using SPSS and Prism 5.0 softwares. A total of 1197 prescriptions were reviewed. Pregabalin prescriptions rate were higher than gabapentin specifically in outpatients clinics ( $P<0.05$ ). Females showed higher rates of using both gabapentin and pregabalin than males ( $P<0.05$ ). In general there was a high rate of gabapentin and pregabalin prescriptions. Further studies need to be done to evaluate the most serious side effects and to control the safety of these prescriptions as well as preventing their misuse.**

**Key words:** Gabapentin, Pregabalin, Indications, Off label.

## INTRODUCTION

### Gabapentin and its uses

Gabapentin (brand names include Neurontin and Horizant) is an anti-epileptic and an anticonvulsant drug

\*Corresponding author. E-mail: [yzhindi@uqu.edu.sa](mailto:yzhindi@uqu.edu.sa).

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(Cheng and Chiou, 2006). It works by affecting the chemicals and nerves inside the body that can cause seizures and some types of pain. Gabapentin has a chemical structure that is derived from the addition of a cyclohexyl group to the backbone of gamma-aminobutyric acid (GABA). Gabapentin has no activity at GABAA or GABAB receptors of GABA uptake carriers of the brain, also Gabapentin can interact with a high-affinity binding site in brain membranes, which has recently been identified as an auxiliary subunit of voltage-sensitive  $Ca^{2+}$  channels. However, the functional mechanisms of gabapentin is unclear and remains under study. Gabapentin crosses several lipid membrane barriers via system L amino acid transporters (Han et al., 2016).

Gabapentin has the ability to prevent pain responses in several animal models of hyperalgesia also it can show efficacy *in-vitro* and *in-vivo* by preventing neuronal death with models of the neurodegenerative disease amyotrophic lateral sclerosis (ALS) (Cheng and Chiou, 2006). It can be found in the form of capsule: 100 mg, 300 mg, 400 mg or in the form of tablet: 300 mg, 600 mg and 800 mg. The label uses of gabapentin are fibromyalgia, nerve pain (neuralgia), pain, peripheral neuropathy, numbness and tingling with pins and needles. Major side effects of gabapentin include dizziness, drowsiness, unsteadiness, memory loss, lack of coordination, difficulty speaking, viral infections, tremors, double vision, fever, unusual eye movements and jerky movements (Han et al., 2016). For the rare but serious possible side effects include suicidality, depression, allergic reaction, severe skin reaction and/or swelling and muscle tissue breakdown (Han et al., 2016).

According to researchers, individuals have reported that abusing gabapentin will produce euphoric effects which are similar to the effects produced by using cannabis and these effects are increasing in sociability, calmness and relaxation (Han et al., 2016). The essential inspiration for abusing gabapentin incorporates amusement, self-damage and self-drug. The abuse of gabapentin can be perilous for a few reasons. At the point when an individual begins on gabapentin by a human services endorse, the prescriber should screen that individual for any unfriendly impacts. Gabapentin has short half-life approximately about 5 to 7 h and the withdrawal symptoms may occur within 1 to 2 days after. The possible signs and symptoms of gabapentin withdrawal include irritability, sweating, nausea, anxiety, confusion, insomnia, increased heart rate, pain and seizures.

### **Pregabalin and its uses**

Pregabalin marketed under the brand name Lyrica is a lipophilic gamma-amino-butyric acid (GABA) analog (Baidya et al., 2011), which have anticonvulsant,

anxiolytic and sleep-modulating properties. It works by binding to the  $\alpha 2\text{-}\delta$  subunit of presynaptic, voltage-dependent calcium channels which they are widely distributed throughout the central nervous system and peripheral nervous system (Baidya et al., 2011). Pregabalin absorption takes one hour and the bioavailability is 90%. When the dose increases, the absorption increases resulting in linear kinetics. The elimination half-life is 5.5 to 6.7 h independent of dose and repeated administration. It is not exposed to hepatic metabolism and is not bound to plasma proteins. Around 98% of the absorbed dose is excreted unchanged in urine. Pregabalin elimination depends on creatinine clearance (CLcr) and it is recommended to reduce half the dose for patients with  $CLcr < 60$  ml/min. Pregabalin is available in tablets dosage form with different doses as 50, 75, 100, 150, 200, 225 and 300 mg. Daily dose can be between 50 to 600 mg/day. Several studies declared that adverse events observed when Pregabalin was taken in overdose range from 800 mg/day to 11,500 mg as a single dose (Baidya et al., 2011). The major label use of pregabalin consist of neuropathic pain, incisional injury, and inflammatory injury and anxiety disorder.

Pregabalin is associated with transient mild to moderate adverse effects which are dose dependent. Less common adverse effects are dry mouth, peripheral edema, blurred vision, weight gain, and inability to concentrate. Pregabalin in acute postoperative pain is used because of the anxiolytic effect and its ability to prevent opioid tolerance (Morrison et al., 2017). Moreover, Pregabalin is considered one of the drugs that can cause dependence on or addiction to, even if the patient is taking it exactly as prescribed; the reason is it produces a relaxed, calm and euphoric sensation. So, when you suddenly stop the drug after chronic use there is potential to develop withdrawal symptoms including difficulty sleeping, nausea, headache and diarrhea (Morrison et al., 2017).

### **Off-label use**

Off-label use, as defined by Health Canada, is the use of a marketed health product outside indications included in the approved product labelling. Off-label use of medications is a common practice in medicine; it is neither restricted to highly specific clinical situations nor to single countries (Boos, 2003). Challenged by diseases without effective treatments or the failure of standard therapies, physicians may try new drug approaches that have some theoretical basis (Gazarian et al., 2006). Off-label drug use does not imply improper or illegal use, and it can provide opportunities to capitalize on a drug's potential effectiveness. However, there are also potentially negative effects of off-label use, which include adverse reactions, liability for pharmaceutical

**Table 1.** Rate of gabapentin and pregabalin prescriptions in both in-patients and outpatients clinics.

Parameter	Gabapentin			Pregabalin		
	In-pateints	Out pateints	Total	In-pateints	Out pateints	Total
Total number of prescriptions	40±6	287±21	327±25	114±34*	756±45*	870±66*
Total number of discharged prescriptions	0	19±13	19±13	0	39±23	39±23

\*( $P < 0.05$ ) pregabalin (in-patient, outpatient and total) vs. gabapentin (in-patient, outpatient and total).

manufacturers and health care practitioners, lack of patient reimbursement for medications purchased for off-label uses and concerns with respect to the illegal promotion, advertising and marketing of off-label uses by the manufacturer (Gazarian et al., 2006). As Haw and Stubbs state, "The use of a medication off label represents an area of potentially increased risk, since the national body that licenses drugs for medicinal use... has not examined the risks or benefits of using the drug in these circumstances" (p. 402) (Haw and Stubbs, 2005). Off-label prescribing and use also have the potential to be ineffective, resulting in wasteful medication use and possibly putting patients at risk.

The off-label uses of Gabapentin include restless legs syndrome, insomnia, diabetic neuropathy, hot flashes-cancer related, amyotrophic lateral sclerosis, bipolar disorder, attention deficit disorder, periodic limb movement disorders of sleep, premenstrual syndrome, migraine headache, drug, and alcohol withdrawal seizures (Wiffen et al., 2017) (Peckham et al., 2017). While the off-label uses of Pregabalin can be for cough, chronic refractory, anxiety disorder, postoperative pain, pruritus, neuropathic or malignancy related, uremic, social anxiety disorder, and vasomotor symptoms associated with menopause (Morrison et al., 2017).

## Aim

The aim of this study was to identify the rate of prescription of Gabapentin and Pregabalin and the rate of prescription in male and female adults. In addition, is identifying the indications for those prescriptions and their percentages.

## METHODS

### Study design

A cross-sectional study was conducted from medical records of in-patients and outpatients clinics from January, 2018 through January, 2019.

### Study setting

The study was conducted at King Abdul-Aziz (Alzaher) Hospital – Makkah.

### Sample size

A total of 1747 prescriptions, 550 total of unaccessible prescriptions and a total of 1197 accessible prescriptions, of which 870 and 327 Pregabalin and Gabapentin prescriptions, respectively.

### Data collection

Data was collected from medical case records.

### Inclusion criteria

Male and female adult ranged from 30 to 55 years old patients under treatment with gabapentin and or/pregabalin.

### Exclusion criteria

This include children, old age, refill prescription and other types of pain medications.

### Data analysis

Data analysis was performed using SPSS and Prism 5.0 software. Values were expressed as means  $\pm$  SD unless otherwise indicated. General linear models were used in the analysis. Repeated measures analysis of variance (Two- way ANOVA) was used in case of indications percentages. t-test with a Bonferroni correction for multiple comparisons was used as a post hoc test. All the tests were two-tailed with the significance level set at  $P < 0.05$ .

### Ethics

Ethical approval was obtained from Umm Al-Qura University Institutional Review Board (IRB) commity UQU- COP-EA-#143914.

## RESULTS

### Rate of gabapentin and pregabalin prescriptions in both in-patients and outpatients clinics/ departments

Table 1 demonstrates the rate of gabapentin and pregabalin prescriptions in both in-patients and outpatients clinics/departments; it appeared that pregabalin prescriptions rate were higher than gabapentin specifically in outpatients clinics (t-test  $P < 0.05$ ). It was a total of 327 and 870 for gabapentin and pregabalin

**Table 2.** Percentage of gabapentin and pregabalin dispensing in each department.

Gabapentin		Pregabalin	
Department	Percentage	Department	Percentage
Urology	0.45	Urology	0.37
Rheumatology	11.16	Rheumatology	3.56
Respiratory	1.34	Respiratory	0.75
Radiology	0.22	Radiology	0.09
Psychiatry	0.22	Psychiatry	0.28
Physical therapy	0.22	Physical therapy	0.37
Orthopedic surgery	1.12	Orthopedic surgery	10.58
Ophthalmology	0.22	Ophthalmology	0.28
Neurosurgery	32.81	Operation rooms	0.19
Neurology	26.79	Neurosurgery	28.18
Nephrology	0.45	Neurology	20.69
Internal medicine	4.24	Nephrology	1.12
General surgery	1.56	Internal medicine	15.45
General practitioner	0.22	Infectious diseases	0.37
ENT surgery	0.45	General surgery	1.69
Endocrinology	1.12	General practitioner	0.37
Emergency	2.90	Gastroenterology	1.69
Dermatology	1.79	ENT Surgery	0.37
Dentist	0.67	Endocrinology	3.75
Cardiology	10.04	Emergency	2.34
		Dermatology	2.43
		Dentist	0.66
		Cardiology	5.99

prescriptions, respectively.

### Rate of gabapentin and pregabalin dispensing in each department

Table 2 demonstrates the percentages of gabapentin and pregabalin dispensing in each department. Gabapentin was prescribed at high percentage in the following clinics: Neurosurgery, neurology and rheumatology with 32.81, 26.79 and 11.16%, respectively. Pregabalin was also prescribed at high rates at neurosurgery and neurology clinics with 28.18 and 20.69%, respectively. In addition, pregabalin was also prescribed at high rates in Internal medicine clinics with 15.45%.

### Female and male rate of prescriptions

Females showed higher rates (~60%) of using both gabapentin and pregabalin than males, moreover there was a higher significant use of gabapentin over pregabalin in females by ~15% (t-test,  $P < 0.05$ ; Figure 1).

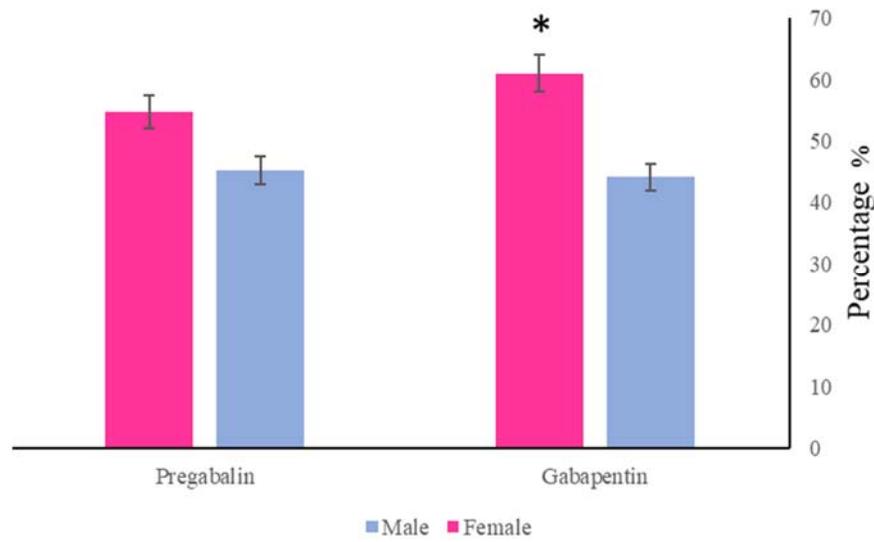
### Percentages of each indication

Table 3 demonstrates the percentages of each indication for gabapentin and pregabalin prescriptions. In general a significant interaction was shown between gabapentin and pregabalin number of prescriptions and each indication, two-way Anova,  $F(1, 13) = 9.518$ ,  $P < 0.05$ .

CNS disorders such as cord compressions, carpal tunnel syndrome and epilepsy were among the highest percentages of prescriptions of 27.83 and 22.76% for gabapentin and pregabalin, respectively. Next was bone disorders such as congenital deformities of the spine and knee constitutions with 24.46 and 27.24% for gabapentin and pregabalin, respectively. However, there was a very large number of prescriptions without any indications written on it with 17.43 and 12.53% for gabapentin and pregabalin, respectively.

### DISCUSSION

The main aim of this study was to investigate the rate of



**Figure 1.** Bar-chart representing males and females percentages of using gabapentin and pregabalin (n=1197; Males=479, Females=712). \*  $P < 0.05$  gabapentin in females vs. pregabalin in females.

**Table 3.** Percentages of each indication for gabapentin and pregabalin prescriptions.

Indication	Gabapentin (%)	Pregabalin (%)
GIT disorders	1.22	1.61
Infection and inflammations	2.14	4.94
CNS disorders	27.83	22.76
Kidney disorders	0.92	1.26
Bone disorders	24.46	27.24
Cardiac disease	8.87	7.59
Psychiatric disorders	0.61	0.92
Trauma	0.31	0.69
Ocular disorders	0.61	0.11
Respiratory disease	0.61	1.26
Endocrine diseases	7.95	12.99
Skin disorders	4.89	4.02
Miscellaneous	2.14	2.07
No indication written	17.43	12.53

prescription of gabapentin and pregabalin notwithstanding the rate of their use in male and female grown-ups. Besides, the indications for those drugs and their rates were identified. A cross-sectional investigation was led; information were collected from restorative medical records from the in-patients and out-patients clinics and/or departments picking a period from January 2018 until January 2019. The investigation secured both male and female grown-up patients somewhere in the range of 30 and 55 years old under treatment with gabapentin as well as pregabalin. A study in North America and parts of

Europe showed that nearly more than half of the patients newly prescribed pregabalin and gabapentin for neuropathic pain were adults (Moore et al., 2014). This elucidates the importance of better understanding of the prevalence and indications of pregabalin and gabapentin in those group of the population. After every single prescriptions was screened, the non-accessible were around 550 prescriptions, while the accessible were around 1197 prescriptions that were chosen relying on the inclusion/exclusion criteria. Via looking through the literature, it was found that this is the first study that

assess the prevalence rate and indications of gabapentin and pregabalin prescriptions in Makkah AlMukarramah, Saudi Arabia.

Results in this study demonstrated that in both in-patients and outpatients pregabalin prescriptions rate were higher than gabapentin specifically in outpatients clinics. Moreover, the rate of prescriptions at the outpatients clinics were significantly higher than the in-patients.

The results also demonstrated the percentage of gabapentin and pregabalin dispensing in each department; It was found that gabapentin was highly prescribed as the most in neurosurgery. Additionally, it was found that females showed higher rates of using both gabapentin and pregabalin than males. To be more precise, there was higher significant use of gabapentin over pregabalin in females by ~15%. A study of retrospective criteria demonstrated that females also used gabapentin more frequently than males, this might be due to the nature of their indications such as after breast cancer surgery and sciatica pain (Fleet et al., 2018; Grice and Mertens, 2008).

It also demonstrated the percentages of each indication for gabapentin and pregabalin prescriptions. The most significant interaction between gabapentin and pregabalin number of prescriptions and each indication were the CNS and bone disorders. However, there was a very large number of prescriptions without any indications written on it. This might indicate a misuse or off-label prescription matter that should be investigated thoroughly in a future study. A scope of ongoing reports have stressed the capability of gabapentin and pregabalin abuse in chosen populaces. Pregabalin was recognized in 12.1% (n = 15) of pee tests from sedative dependent subjects going to a German habit facility. None of these patients were experiencing any of the signs for pregabalin endorsing, with most having confirmed that they had obtained pregabalin illegally while being very much aware that the particle was excluded in the standard medication checking framework (Grosshans et al., 2013). One of the restrictions in this investigation that we could not most likely know any conceivable symptoms of those medications in the examination populace, along these lines we were not ready to survey this issue. Another constraint of the investigation was that it did not locate some other sedatives or opiates that were recommended with gabapentin or pregabalin; in this manner, the impact of gabapentin and pregabalin in diminishing the utilization of different sedatives/ painkillers or not could not be surveyed.

It is intriguing to take note of the fact that gabapentin and pregabalin has been endorsed in Canada and the USA since 2005, and endorsement by the European Commission to treat summed up uneasiness issue was gotten in 2006 (Canadian Agency for Drugs and Technologies in Health (CADTH), 2012). However, the discussion with respect to mishandle and reliance did not

show up in the medicinal writing before 2010 (Filipetto et al., 2010). Essentially, staying physician endorsed drugs with abuse potential (for example benzodiazepines; z-hypnotics) were considered 'safe' for a long time before their addictive risk levels were identified (Filipetto et al., 2010). This might be a result of pre-advertising clinical preliminaries commonly including the organization of deliberately controlled, every day helpful measurements. Besides, it is a notable shortcoming of these preliminaries that subjects with compulsion issue are rejected (Schwan et al., 2010). As an outcome, the genuine capability of abuse of the file atom will be all the more appropriately refreshing just when countless, who will include powerless people, are presented to the medication. Therefore, a superior appraisal/clarification of gabapentin and pregabalin abuse potential dimensions is without a doubt of intrigue. Truth be told, interestingly with maltreatment obligation information, gabapentin and pregabalin may conceivably speak to a significant resource in the pharmacological collection of habit prescription (Schwan et al., 2010). Since these drugs are generally endorsed drugs, wellbeing experts ought to be very much aware of both the potential dangers for their abuse and the related cessation side effects. Doctors considering endorsing gabapentin and pregabalin for neurological/mental or pain scatters should cautiously assess a conceivable past history of medication misuse. Moreover, they ought to have the capacity to immediately recognize indications of pregabalin/gabapentin abuse, while giving help with decreasing the prescription (Filipetto et al., 2010). Further exact examinations with gabapentin and pregabalin ought to be empowered, concentrating on a superior appraisal of their addictive obligation levels over a scope of doses and in people with a past substance abuse history.

## Conclusion

A high rate of gabapentin and pregabalin prescriptions has been seen in general. Further studies need to be done to evaluate the most serious side effects and to control the safety of these prescriptions as well as preventing their misuse.

## Recommendations

One of the vital bearing of this examination is to guarantee the wellbeing of patient from the unsafe unfavorable impacts that may happen from utilizing gabapentin and pregabalin; the checking procedure is favored likewise to help in decreasing the endorsing of gabapentin and pregabalin as could reasonably be expected or to locate an elective analgesics with less hazard to conquer any conceivable extreme antagonistic impacts. Gabapentin and pregabalin both have the

capability of being manhandled; numerous systems ought to be considered for this; independently persistent instructive intercessions about the right use, portion, term, will be useful to control the abuse. To make new rules for gabapentin and pregabalin use and organization that will be useful and can be actualized inside medical clinics.

## Limitations

Limitations of this study include:

1. Not all prescription were clear with the precise diagnosis.
2. Some errors were detected in entries at the pharmacy, especially quantities of issued medicines and some double entries.
3. Inability to identify any side effects or other medications used by the study populations from the medical records.

## REFERENCES

- Baidya DK, Agarwal A, Khanna P, Arora MK (2011). Pregabalin in acute and chronic pain. *Journal of anaesthesiology, clinical pharmacology* 27(3):307.
- Boos J (2003). Off label use-label off use. *Annals of Oncology* 14:1-5.
- Canadian Agency for Drugs and Technologies in Health (CADTH) (2012). Abuse and misuse potential of pregabalin: A review of the clinical evidence; Context and policy issues. [http://dpic.org/sites/default/files/PregabalinAbuse\\_CADTH\\_24Apr2012.pdf](http://dpic.org/sites/default/files/PregabalinAbuse_CADTH_24Apr2012.pdf). (Accessed 20 March 2019).
- Cheng JK, Chiou LC (2006). Mechanisms of the antinociceptive action of gabapentin. *Journal of Pharmacological Sciences*, 0602100009-0602100009.
- Filipetto FA, Zipp CP, Coren JS (2010). Potential for pregabalin abuse or diversion after past drug-seeking behavior. *The Journal of the American Osteopathic Association* 110(10):605-607.
- Fleet JL, Dixon SN, Kuwornu PJ, Dev VK, Montero-Odasso M, Burneo J, Garg AX (2018). Gabapentin dose and the 30-day risk of altered mental status in older adults: A retrospective population-based study. *PLoS One* 13(3):e0193134.
- Gazarian M, Kelly M, McPhee JR, Graudins LV, Ward RL, Campbell TJ (2006). Off-label use of medicines: consensus recommendations for evaluating appropriateness. *Medical Journal of Australia* 185(10):544-548.
- Grice GR, Mertens MK (2008). Gabapentin as a potential option for treatment of sciatica. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* 28(3):397-402.
- Grosshans M, Lemenager T, Vollmert C, Kaemmerer N, Schreiner R, Mutschler J, Hermann D (2013). Pregabalin abuse among opiate addicted patients. *European Journal of Clinical Pharmacology* 69(12):2021-2025.
- Han C, Li X, Jiang H, Ma J, Ma X (2016). The use of gabapentin in the management of postoperative pain after total hip arthroplasty: a meta-analysis of randomised controlled trials. *Journal of Orthopaedic Surgery and Research* 11(1):79.
- Haw C, Stubbs J (2005). A survey of the off-label use of mood stabilizers in a large psychiatric hospital. *Journal of Psychopharmacology* 19(4):402-407.
- Moore A, Wiffen P, Kalso E (2014). Antiepileptic drugs for neuropathic pain and fibromyalgia. *Jama* 312(2):182-183.
- Morrison EE, Sandilands EA, Webb DJ (2017). Gabapentin and pregabalin: do the benefits outweigh the harms?. *Journal of the Royal College of Physicians of Edinburgh* 47(4):310-313.
- Peckham AM, Fairman KA, Sclar DA (2017). Prevalence of Gabapentin Abuse: Comparison with Agents with Known Abuse Potential in a Commercially Insured US Population. *Journal of Clinical Drug Investigation* 37:763.
- Schwan S, Sundström A, Stjernberg E, Hallberg E, Hallberg P (2010). A signal for an abuse liability for pregabalin—results from the Swedish spontaneous adverse drug reaction reporting system. *European Journal of Clinical Pharmacology* 66(9):947-953.
- Wiffen PJ, Derry S, Bell RF, Rice ASC, Tölle TR, Phillips T, Moore RA (2017). Gabapentin for chronic neuropathic pain in adults. *Cochrane Database of Systematic Reviews* doi: 10.1002/14651858.CD007938.pub4.

*Full Length Research Paper*

# Pharmaceutical polymorphisms and its influence on the dissolution profile of two pioglitazone brands

Eduardo Lozano Guzmán<sup>1\*</sup>, Saúl Alejandro Aguirre Rentería<sup>1</sup>, María Guadalupe Nieto Pescador<sup>1</sup>, Maribel Cervantes Flores<sup>1</sup>, Ángel Antonio Vértiz Hernández<sup>2</sup> and Francisco Javier Moreno Cruz<sup>1</sup>

<sup>1</sup>Pharmacognosy Laboratory, Faculty of Chemical Sciences, Juarez Durango State University, Mexico.

<sup>2</sup>Technology and health laboratory, Academic Coordination Altiplano Region, Autonomy University of San Luis, Potosi, Mexico.

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The aim of this work is to identify the presence of pharmaceutical polymorphisms in two brands of pioglitazone and evaluate their influence on the dissolution of tablets *in vitro*. The presence of polymorphisms was determined by Raman spectrometry and Infrared spectrometry. To relate the presence of these polymorphisms with the dissolution capacity of the drug (bioavailability *in vitro*), the dissolution profile of the drugs was determined through the development and implementation of a high resolution liquid chromatography (HPLC) method using a detector UV-vis. Two displacements were detected in Raman spectroscopy, suggesting an alteration in the crystalline structure of the test drug in relation to the reference drug. Significant differences were also found in the dissolution profile evaluated by the dissolution factor "f2", which could be explained by the presence of these polymorphisms. The presence of pharmaceutical polymorphisms can lead to the alteration of the processes of absorption and metabolism *in vivo* and therefore, alteration in the therapeutic effect.

**Key words:** Pharmaceutical polymorphism, dissolution, pioglitazone, high resolution liquid chromatography (HPLC), Raman spectrometry.

## INTRODUCTION

The absorption of a drug in solid dosage form orally depends on various processes including drug dissolution and transport within the body. The processes involved in the absorption, mainly the release and dissolution are critical to achieve a good therapeutic effect because the bioavailability of a drug depends on the degree and speed of dissolution and its absorption. These processes

depend on the physicochemical characteristics of the drugs, which include the crystalline forms. Defects in the elaboration of medications can alter the crystalline formations that later will alter the absorption processes and, therefore, the efficacy of the medication (De Salvi, 2011). These alterations in the structure of a solid are called polymorphisms. Polymorphism is defined as the

\*Corresponding author. E-mail: [elozano@ujed.mx](mailto:elozano@ujed.mx).

ability of a solid material to exist in two or more crystalline forms that present different conformational arrangements (Sánchez et al., 2007). More than 600 active ingredients and excipients are known that are used to manufacture medicines which have the ability to acquire different crystalline forms. The formation of different crystalline forms depends on the quality in the manufacturing process of the drug (Islán and Montes, 2006), the temperature used during manufacturing, the use of solvents, the evaporation process and the presence of contaminants, among other factors (Lara and Lopez, 2017). The presence of one or another crystalline form can directly influence the solubility of the drug and it is known that the presence of polymorphic structures in drugs can alter their bioavailability and half-life and therefore have a different therapeutic effect (Hiendrawan et al., 2017; Zhou et al., 2018)). This is why *in vitro* dissolution profiles can help predict the behavior of drugs *in vivo*.

In general, polymorphs can be studied by X-ray diffraction, thermal analysis, vibrational spectroscopy, nuclear magnetic resonance in solid state and microscopy. However, Raman spectroscopy and Infra-red analysis have now been used because they present better resolution (Prohens and Puigjaner, 2007; Du and Xue, 2016). The Raman technique is a type of molecular vibrational spectroscopy which bases its operation on the Raman effect consisting of an inelastic dispersion of a photon by the molecules that make up the material. Raman spectroscopy has great importance in the study of objects of cultural interest thanks to its high specificity, its non-destructive nature on certain materials and micro destructive in others; it is also non-invasive and has an excellent spatial resolution (García, 2013). This technique, in combination with infrared spectrometry, represents an excellent option for the study of the crystalline configuration of drugs.

On the other hand, pioglitazone is a drug belonging to the family of thiazolidinediones used for the control of diabetes mellitus type 2. Of all the drugs that formed this family only pioglitazone remains on the market, although it has already been removed from some countries like France due to its adverse effects, mainly liver damage. However, in Mexico and many other countries it is still manufactured and marketed in different brands. Reports on changes in therapeutic effects due to the presence of polymorphisms in diabetic patients were not found. The aim of the present work was to determine the relationship between the dissolution profile of two pioglitazone brands and the presence of pharmaceutical polymorphisms.

## MATERIALS AND METHODS

### Reagents

Acetonitrile grade high resolution liquid chromatography (HPLC)

brand J.T. Baker lot 9017-03 C. Distilled water in the same laboratory, CTR brand reagent grade acetic acid and ethyl acetate HPLC brand J.T. Baker lot 604033. The reference listed drug was Pioglitazone hydrochloride in 15 mg tablets of Zactos® manufactured by Takeda - Japan and distributed in Mexico by Eli Lilly, lot C410501C. The test drug was Pioglitazone hydrochloride in 15 mg tablets issued as a generic drug, (lot 15E087). This drug is one of the most consumed by the diabetic population due to its low price. A HPLC reference standard was used, manufactured by Aarti Drugs Limited, Lot PIO / 10100105 ES-DA-022.

### Development and validation of the chromatographic method

An Agilent model 1100 chromatograph with UV-vis detector VWD G1314A ( $\lambda = 230$  nm) with quaternary pump G1311A and an Agilent Eclipse XDB-C18 4.6x150 mm and 5 $\mu$ m column were used. The methodology used was developed based on modifications made to the work reported by Vertiz et al. (2014). A solution of 0.1% acetic acid and acetonitrile in a 50:50% v/v ratio was used as the mobile phase. The following concentrations were established as working range: 80, 200, 400, 800, 1000, 1300, 1600, 2000 and 2600 ng/ml. The mobile phase was composed of acetic acid (ac) 0.1%/Acetonitrile 50/50% v/v with isocratic elution at a flow of 1.2 ml/min. 50  $\mu$ l was injected. The validation of the chromatographic method was carried out based on the provisions of the Mexican standard NON-177-SSA1-2013 that includes the following parameters:

- (i) Linearity: three calibration curves were prepared and analyzed into the working range. The average of the Pearson regression coefficient  $r \geq 0.99$  was rated as acceptable. Precision: The coefficient of variation -C.V.- (standard deviation / average) was calculated for the analytical responses of each concentration level of the work range. It was rated as acceptable when C.V.  $\leq 2\%$ . Further, intraday precision was determined by the analysis of 5 repetitions of standard concentration samples known (high and low) on the same day. The interday precision was determined by the analysis of 3 known standard concentration samples (high and low) analyzed in three successive days.
- (ii) Accuracy: Using the precision data (peak areas), the concentration in each level of the curve and standard concentration samples known (high and low) was calculated by the corresponding equation and the Relative Error expressed as Relative Standard Deviation ( $RSD = \frac{|\text{Known Concentration} - \text{Calculated Concentration}|}{\text{Known Concentration}}$ ) was determined and expressed as percentage. The curve was qualified as exact when at least 75% of the levels met criterion  $RSD \leq 2.0\%$ . If a level failed in this criterion, it was eliminated from the curve and the curve was recalculated.
- (iii) Sensitivity: the limit of quantification (sensitivity) was established as the lowest concentration of the working range (80 ng/ml)
- (iv) Stability of the sample during processing: a series of three samples was analyzed at the beginning and end of the process and the initial and final signals were compared. The samples were rated as stable if the absolute difference of the average of the percentage quantified in the initial and final analysis was  $\leq 3\%$ .
- (v) Stability in solution: To build calibration curves and quality solutions, a mother solution and secondary solutions were used, which were prepared in mobile phase and stored for not more than a week. To demonstrate its stability, the concentration of the solutions was analyzed at the time of preparation and compared against the concentration analyzed after one week. They were taken as stable if the  $RSD \leq 5\%$ . All the solutions were stored in refrigeration ( $5 \pm 2^\circ\text{C}$ ).
- (vi) Selectivity: it was verified that there were no interferences in

the analytical answers. In case of interferences, these should not be greater than 3% of the minor response of the working range. (vii) Equipment suitability: For each analytical run, the injector, the pump and the column were evaluated by injecting a solution of known standard concentration in triplicate. The performance of the pump and column was rated as acceptable if the average retention time of the three injections was less than 5%. The injector was rated as acceptable if the average of the analytical responses of the three injections was less than 5%. To evaluate the column, calculate the number of theoretical plates (N) based on the average of the retention times and the peak width at the average height. The column was rated as acceptable when  $N \geq 2000$ .

(viii) Quality control: A calibration curve was prepared for analytical run. The concentrations of the samples were calculated by the corresponding equation on each work day. Additionally, quality solutions were prepared consisting of three levels of known concentration of reference standard (high, medium and low) prepared in triplicate. These were distributed homogeneously in the analysis lot. Subsequently, the concentration of each of them interpolated in the corresponding calibration curve was calculated. The analytical run was qualified as acceptable when the calibration curve met the linearity criteria and the control samples met the accuracy criteria.

### Sample dissolution

A Hanson Research Corp. Model 64-100-121 pallet dissolutor was used. The stirring speed was set at 50 r.p.m. The drugs were dissolved in three different solutions: A) 0.1 N hydrochloric acid (pH 1.2) that simulates gastric conditions. B) Phosphate buffer solution of pH 4.5 and C) Phosphate solution of pH 6.8 simulating intestinal fluid. 6 tablets of each drug were separately dissolved in each of the solutions. An aliquot of 1 ml was taken from time zero to 45 min every 3 min and diluted in a 1:10 ratio to be analyzed in the chromatograph.

### Equality criteria

The similarity factor  $f_2$  was used to determine the equality between the dissolution profiles. Both the NOM-177 and the FDA (Guidance Drugs) define  $f_2$  as a transformation of the logarithmic reciprocal square root of the sum of the error and it is an adequate instrument to measure the similarity in the percent dissolution.

$$f_2 = \log \left[ \frac{100}{\sqrt{1 + \left(\frac{1}{t}\right) S(Ri - Pi)^2}} \right]$$

$t$  = number of sampling times.

$Ri$  = Average of the dissolved percentage of the reference medicine in the "i" sampling time.

$Pi$  = Average of the dissolved percentage of the test drug in the "i" sampling time.

The drugs have a similar rate of dissolution when the similarity factor  $f_2 \geq 50$ .

### Raman and infrared spectrometry

The Raman and Infrared techniques tend to complement one

another, and are ideal for studying pharmaceutical polymorphisms. Raman is generally better at identifying non-polar functional groups, while infrared behaves better at polar molecular vibrations (Vértiz et al., 2014). Raman spectrometry is based on the inelastic shock of the radiation incident on a molecule, that is, the scattered radiation is of different wavelength than the incident radiation. The radiation consists of a beam of monochromatic light that goes from the UV to the infrared (García, 2013). The vibration of the functional groups of a compound subjected to Raman produces a characteristic spectrum that can be compared with other compounds to verify if they are similar or not (Aubuchon and Gracia-Del Rió, 2018). An Ocean Optics ® model QE65000 was used with a controlled power of 578 mW and an integration time of 2 s. The equipment has a 0.922 intensity laser source at a wavelength of 785 nm and a power of 499 mW with a high sensitivity Hamamatsu S7031-1006 detector. The Spectra Suite ® software was used for signal processing. Samples for Raman analysis consisted of one tablet of each drug extracted from the package and directly subjected to analysis. A sample of the HPLC grade reference standard of purity was also subjected to analysis. The spectra were compared with each other to determine conformational similarities.

On the other hand, infrared (IR) spectrometry is based on the radiation absorbed by the molecules in vibration. An ABB3000 model 3000 medium infrared machine with a range of 450-4000  $\text{cm}^{-1}$  was used for the analysis. A tablet of medicine was taken and pulverized in Agate mortar by adding KBr and then compressed to obtain a uniform transparent glass that was subjected to analysis. The spectra of each medication (test and reference) and the reference standard were compared to determine similarities.

## RESULTS AND DISCUSSION

### Chromatographic method

The method met all the acceptance criteria and was therefore classified as reliable, precise and exact for the established work range. The curves showed that Pearson regression coefficient  $r = 0.9955$ ; Coefficient of variation C.V. = 6.05% and Relative standard deviation R.S.D. = 5.94. The stability was also satisfactory and the samples resisted at least two freeze-thaw cycles.

### Dissolution profile

Pioglitazone dissolves better in acid media, so the dissolution profiles in solutions B and C were inconclusive due to the low solubility presented by both brands. However, in the solution, both drugs (test and reference) present a solution of more than 85% in the first 15 min, so they can be considered as rapidly dissolving drugs. However, the similarity factor  $f_2$  was equal to 25.1, which means that there are significant differences between the test and reference medicine. In Figure 1 it can be seen that the test drug has lower dissolving power; the concentrations in the test drug are lower at any time. Additionally, Figure 2 shows the dissolution profiles in the three solutions tested. As can be seen, the solubility of the drug in B and C is very low.

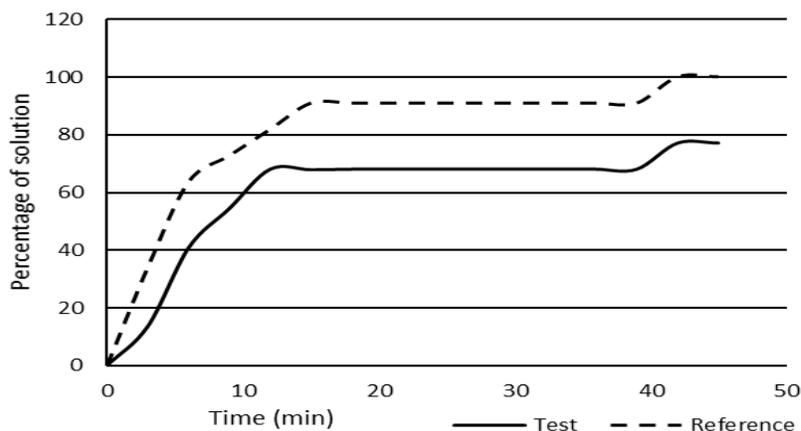


Figure 1. Dissolution profiles of test and reference medicine in solution A.

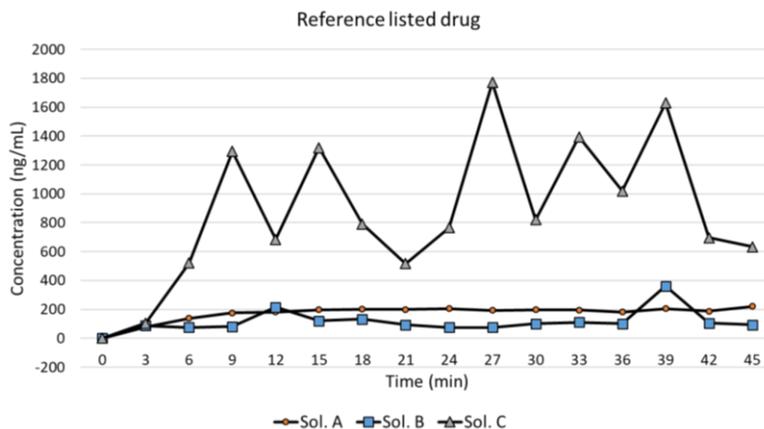


Figure 2. Dissolutions profiles in solutions A, B and C.

**Raman spectrometry**

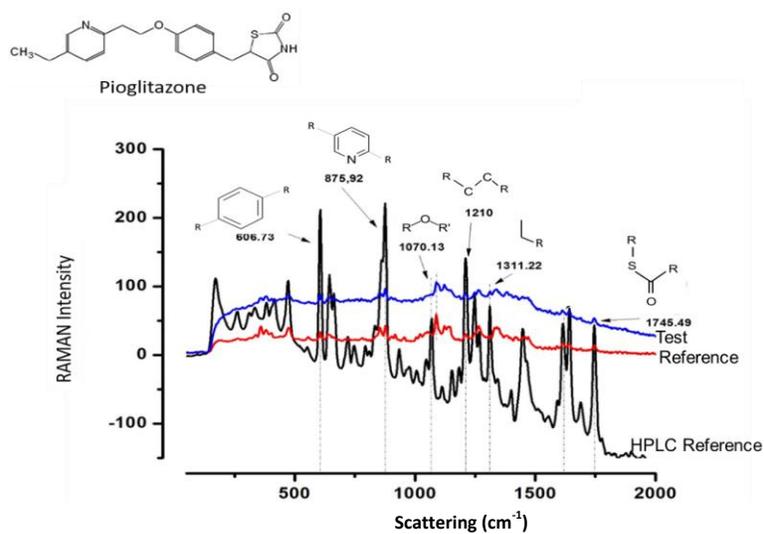
The intensity in the signals of the spectra of the test and reference medicine was lower than the intensity observed in the reference substance. This can be attributed to the presence of the excipients present in the drugs and which are not found in the reference substance due to its purity. However, the spectrum shows two anomalies in the dispersion of the response at 1070.13 cm<sup>-1</sup> and 1311.22 as shown in Figure 3. This response suggests the presence of different configuration between the test and reference substance' that is it suggests the presence of pharmaceutical polymorphism.

In addition, the displacement at 606.73 cm<sup>-1</sup> presents an intense signal in the reference substance but not in the test drug. On the contrary, in displacements 875.92, 1070.13 and 1745.49 cm<sup>-1</sup> the same signals are present in the test and reference medicine, so it could be

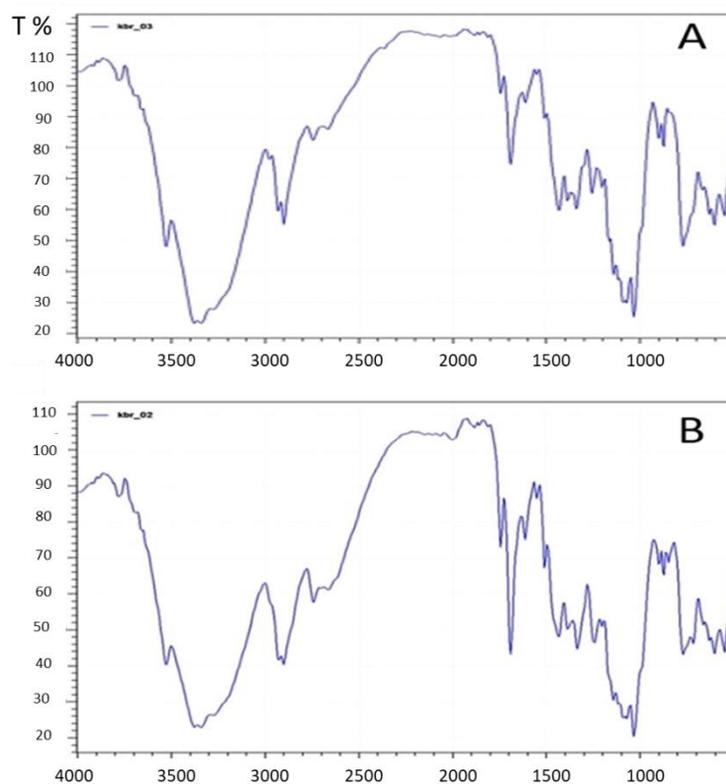
concluded that these three functional groups do not differ from each other. Raman spectroscopy is based on the vibration responses of functional groups. Each functional group presents a characteristic displacement. The fact that there are anomalies in these responses suggests the presence of changes in the functional groups in the structural conformation of the molecule. In the infrared analysis, there are no differences in the signals of the test and reference medicines, as shown in Figure 4.

**DISCUSSION**

Two crystalline forms of pioglitazone hydrochloride are currently known, referred to as Form I and Form II (Tao-Zhao et al., 2013). It has been found that pioglitazone hydrochloride form II is identical to pioglitazone base. However, form I of pioglitazone hydrochloride is a



**Figure 3.** RAMAN spectrometry of the functional groups of two brands of pioglitazone and reference substances.



**Figure 4.** Infrared spectroscopy of the reference drugs (A) and tests (B).

conglomerate that is routinely used in the manufacture of the drug. Even when the two forms could be present in the samples, they interconvert *in vivo*. Until now no

differences have been detected in its pharmacokinetics (Chengcheng and Adam, 2017). The results obtained in our Raman analysis and in the solubility profiles suggest

the presence of polymorphisms or variations in the crystalline conformations of the pioglitazone hydrochloride of the test drug, but they do not seem to have any relation with the common Form I and II of pioglitazone. The formation of crystals during the tableting process can be due to several factors, such as the method used (sublimation, evaporation of the solvent, heat treatment, etc.). The use of different additives during the crystallization of pioglitazone hydrochloride also influences the power of solubility because a crystalline configuration can lead to a greater porosity and decrease in density, increasing solubility, and modifying absorption in the stomach (Sachinkumar et al., 2012).

Many drugs of solid form can exist in various crystalline forms and have variable physicochemical properties. These variations can influence such a way that a medicine considered as bio-interchangeable changes to be non-bio-interchangeable because it presents different crystalline forms than the original medicine (Zhou et al., 2018; Fuentes et al., 2006). When a drug presents polymorphisms it is recommended to choose the formulation that produces the most stable form, however, the question always arises: are all the polymorphisms known? Do we know the most stable form? (Garland, 2007). At present, the dissolution profile is taken as a predictive tool for *in vivo* product development and its biopharmaceutical properties (Gonzalez et al., 2015). Therefore, it is necessary to analyze more closely the crystalline configurations and co-relate them with their solubility to prevent unwanted pharmacokinetics. Rifampicin, for example, has a very variable bioavailability due to the presence of polymorphisms. Because of this, WHO already recommends using only those formulations whose dissolution has been satisfactorily tested. Phenylbutazone, verapamil, nitrofurantoin and ampicillin are other examples (Sharman et al., 2011).

Sugita et al. (2014) indicated that for class II drugs in the Biopharmaceutical Classification System with low solubility and high permeability, the solubility and/or dissolution rate in the gastrointestinal tract are considered the principal factors limiting oral absorption. In this study, particles smaller than 1 mm help better solubility, as presented in the reference listed drug. The use of hydroxypropyl cellulose as part of the excipients helps pioglitazone to have a better solubility. It must be considered then, that apart from the modified structures found in our study, the excipients used in one or the other drugs may also influence the determined solubility profile.

The greater solubility of pioglitazone in acidic media is directly related to its polar structure and its thermodynamic properties. Tao et al. (2013) indicated that the solubility of pioglitazone hydrochloride (Form I) increased in methanol, ethanol, 1-propanol, acetic acid, and *N,N*-dimethylacetamide with increasing temperature, because of the polarity of the solubility system. Finally, it is necessary to highlight that in the infrared analysis no

anomalies were detected between the two drugs analyzed. The reason why the results in Raman and Infrared differ is not very clear and should be the subject of a detailed study in the future.

## Conclusion

The RAMAN spectrometry showed at least two anomalies in the configuration of the test drug, which could be related to the difference in the dissolution profile found. This may have consequences on the bioavailability of the drug in patients who are still using pioglitazone hydrochloride for diabetic control.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Aubuchon S, Gracia-Del Río C (2018). Determinación mediante DSC y DSC-Raman de polimorfismos en principios activos farmacéuticos. [Report], XIV Reunión Anual de la Asociación Argentina de Cristalografía, Argentina.
- Chengcheng Z, Adam JM (2017). A Newly Discovered Racemic Compound of Pioglitazone Hydrochloride Is More Stable than the Commercial Conglomerate. *Crystal Growth and Design* 17(2):414-417. <https://doi.org/10.1021/acs.cgd.6b01638>
- De Salvi BS (2011). Polimorfismo em medicamentos genéricos e similares. Master Thesis, Universidade Estadual Paulista, Brazil.
- Du Y, Xue J (2016). Investigation of polymorphism and cocrystallization of active pharmaceutical ingredients using vibrational spectroscopic techniques. *Current Pharmaceutical Design* 22(32):4917-4928.
- Fuentes NI, Rubio CK, Hernández PL, Portilla MB, Aguilar AE (2006). Estudios de perfiles de disolución, calorimetría diferencial de barrido y tamaño de partícula como elementos para determinar la calidad de materias primas. *Revista Mexicana de Ciencias Farmacéuticas* 37(4):43-57.
- García BM (2013). Desarrollo y aplicación de la espectroscopia Raman amplificada en superficie (sers) para estudio no destructivo de pintura novohispana. [Thesis] Universidad Nacional Autónoma de México. México.
- Garland R (2007). Pharmaceutical polymorphism. *Cuadernos Médico Sociales*, Chile 47(3):191-199.
- Gonzalez MA, Castro A, Melendez L (2015). Identificación de las estructuras polimórficas en comprimidos comerciales de glibenclámda. *Revista Iberoamericana de Producción Académica y Gestión Educativa* 2(4):1-16.
- Hiendrawan S, Widjojokusumo E, Veriansyah B, Tjandrawinata RR (2017). Pharmaceutical salts of carvedilol: polymorphism and physicochemical properties. *AAPS PharmSciTech* 18(4):1417-1425. <http://doi:10.1208/s12249-016-0616-x>
- Islán ÁPM, Montes EM (2006). Polimorfismo farmacéutico: repercusiones industriales y farmacocinéticas. *Offarm: farmacia y sociedad* 25:94-100.
- Lara JC, López AB (2017). Importancia de las sales orgánicas en la industria farmacéutica. *Revista Mexicana de Ciencias Farmacéuticas* 48(1):18-42.
- NOM-177-SSA1-2013. Official Mexican Standard that establishes the tests and procedures to demonstrate that a medicine is interchangeable.
- Prohens R, Puigjaner C (2007). Polimorfismo en la industria

- farmacéutica. *El Farmacéutico* 373:58-68.
- Sachinkumar P, Atmaram P, Sunit KS (2012). Effect of Additives on the Physicochemical and Drug Release Properties of Pioglitazone Hydrochloride Spherical Agglomerates. *Tropical Journal of Pharmaceutical Research* 11(1):18-27. <http://dx.doi.org/10.4314/tjpr.v11i1.3>
- Sánchez E, Jung H, Yépez L, Hernández AV (2007). Relevancia del polimorfismo en el área farmacéutica. *Revista Mexicana de Ciencias Farmaceuticas* 38(2):57-76.
- Sharma A, Mishra R, Tandon P (2011). Polymorphism in Pharmaceutical Compounds. *Advancements and Futuristic Trends in Material Science* pp. 39-48.
- Sugita M, Kataoka M, Sugihara M, Takeuchi S, Yamashita S (2014). Effect of excipients on the particle size of precipitated pioglitazone in the gastrointestinal tract: Impact on bioequivalence. *The AAPS Journal* 16(5):1119-1127. <http://doi:10.1208/s12248-014-9646-z>
- Tao M, Sun H, Wang Z, Cui P, Wang J (2013). Correlation of solubility of pioglitazone hydrochloride in different binary solvents. *Fluid Phase Equilibria* 352:14-21. <https://doi.org/10.1016/J.Fluid.2013.05.008>.
- Tao-Zhao W, Junbo G, Hongxun H, Jingkang W (2013). Determination of the Solubility, Dissolution Enthalpy and Entropy of Pioglitazone Hydrochloride (Form II) in Different Pure Solvents. *Industrial and Engineering Chemistry Research* 52:3036-3041.
- Vértiz BJ, Ávila R, Terrones R, Camacho LA, Rangel LA, Campos CI, Vértiz HA (2014). Clorhidrato de pioglitazona: una perspectiva analítico-instrumental por espectroscopia Raman y HPLC. *Revista de Investigación Clínica* 66:351-358.
- Zhou Y, Wang J, Xiao Y, Wang T, Huang X (2018). The Effects of Polymorphism on Physicochemical Properties and Pharmacodynamics of Solid Drugs. *Current Pharmaceutical Design* 24(21):2375-2382. <https://doi.org/10.2174/1381612824666180515155425>

*Full Length Research Paper*

# Evaluation of hypoglycemic activity and safety of *Carica papaya* seed extracts in alloxan-induced diabetic mice

Samuel P. Kagame<sup>1, 2, 3\*</sup>, Nicholas Mwikwabe<sup>1</sup>, Peter Mwitari<sup>1</sup> and Moses Gichua<sup>2</sup>

<sup>1</sup>Kenya Medical Research Institute, Center for Traditional Medicine and Drugs Research, P. O. Box 54840 00200, Nairobi, Kenya.

<sup>2</sup>Jomo Kenyatta University of Agriculture and Technology P. O. Box GPO 62000 Nairobi, Kenya.

<sup>3</sup>Department of Botany, Faculty of Pure and Applied Sciences, School of Biological Science, JKUAT, Kenya.

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***Carica papaya* is used for the treatment of many diseases such as stomach upsets, diarrhoea, bilharzia, diabetes. The study aims to evaluate the safety and hypoglycemic potential effect of *Carica papaya* seeds on alloxan induced diabetic mice. DCM extracts were examined for the presence of terpenoids, flavonoids, steroids, phenols, tannins and alkaloids. Toxicity profile was established at 5, 50, 200, 300 and 2000 mg/kg for both water and methanol extracts. 3 female mice per dosage were given the crude extracts once and observed for 14 days; their weights were recorded twice a week. The mortality rate was 0%. Solvents used to prepare test extracts included methanol, Dichloromethane (DCM) distilled water and phosphate buffer solution. Mice were induced with diabetic by alloxan IP injection 150 mg/kg b.w and fasted overnight; they were allowed to drink 5% glucose solution to overcome drug induced hypoglycemia. The diabetic mice (>8 mmol/dl) were treated with 100 and 500 mg/kg of water and methanol extract, standard drug metformin (100 mg/kg b.w) and normal saline for 10 days, each group consisting of 5 mice each. Blood samples of 0.1 ml were collected from tail tip using strips; glucose concentration was tested using a glucometer. All data were analyzed and computed by SPSS and presented as mean ± standard deviation (SD).**

**Key words:** Diabetes, hypoglycemic, alloxan, *Carica papaya*.

## INTRODUCTION

Global report on diabetes demonstrates that the number of adults living with diabetes has almost quadrupled since 1980 to 422 million adults (Roberts et al., 2012). This

dramatic rise is largely due to the rise in type 2 diabetes and factors driving it include overweight and obesity. In 2012 alone diabetes caused rise is 1.5 million deaths. Its

\*Corresponding author. E-mail: kagamesam@gmail.com. Tel: +254-722-145649.

complications can lead to heart attack, stroke, blindness, kidney failure and lower limb amputation (Kirigia et al., 2009). The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. Diabetes prevalence has been rising more rapidly in middle- and low-income countries. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation (Kirigia et al., 2009).

In 2015, an estimated 1.6 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributable to high blood glucose in 2012. Almost half of all deaths attributable to high blood glucose occur before the age of 70 years (Roberts et al., 2012). WHO projects that diabetes will be the seventh leading cause of death in 2030. Diabetes can be treated and its consequences avoided or delayed with diet, physical activity, medication and regular screening and treatment for complications. In April 2016, WHO published the Global report on diabetes, which calls for action to reduce exposure to the known risk factors for type 2 diabetes and to improve access to and quality of care for people with all forms of diabetes. According to the International Diabetes Foundation estimates, diabetes mellitus affects about 246 million people worldwide. By the year 2000, approximately 7.1 million Africans were reported to be suffering from diabetes (Motala, 2002). This number is expected to rise to 552 million by 2030 (IDF, 2011). In some rural parts of the country such as Nyeri and Kilifi, the prevalence is as high as 11.6% and above 20% among the richer families in the major urban centers (Chege, 2010).

The estimated total economic cost of diagnosed diabetes mellitus in 2012 was \$245 billion. This estimate is a representative of the substantial burden that diabetes mellitus imposes in the world (Yang and Colditz, 2015). There is a steady increase in the prevalence of diabetes mellitus since much of the population cannot afford costs associated with the management of this disease and lack information on symptoms for diagnosis and management (IDF, 2011). Hence, there is a need to search for potential safe and cost effective anti-hyperglycemic agents.

*C. papaya*, a tropical plant believed to have originated in Southern Mexico and Central America is an evergreen shrub, small tree that grows best in full sun to light shade. Its variety includes: Kamiha, Mexican red, Mexican yellow, solo, sunrise solo, sunset solo, vista solo and Waimanalo solo (Parle and Gurdita, 2011). It has been used locally in the treatment of urinary tract infections among other infections (Aliyu, 2006; Akahet et al., 1997; Anaga and Onehi, 2010; Burkill, 1985; Chinoy et al., 1995; Chinoy and Padman, 1996; Parle and Gurdita, 2011; Sidi-Aliyu, 2006). *C. papaya* is cultivated for its edible ripe fruit; its juice is a popular beverage, and its young leaves, shoots, and fruits are cooked as vegetables (Aravind et al., 2013). This study aimed to establish the hypoglycemic activity of *Carica papaya* seeds extract, test for safety of

the extracts and its effects on the blood glucose concentration. This study also aims to conduct the phytochemical screening of the *Carica papaya* seeds extract.

## STUDY SITE AND DESIGN

### Test drug and chemicals

A single injection of alloxan monohydrate (Alloxan, Sigma Aldrich, USA) was used for diabetic induction in the experimental animals at a dose of 150 mg/kg body weight intraperitoneally. Standard drug used was Metformin (100 mg/kg b.w.). Solvents used to prepare test extracts included methanol, distilled water and phosphate buffer solution.

### Animals

A total of 50 mice (15 mice for acute oral toxicity and 35 mice for hypoglycemic studies) were used. Male *Swiss albino* mice (6 weeks old) from the KEMRI animal facility and weighing  $20 \pm 2$  g were moved to an experimental room for acclimatization for one week prior to the experiment. Five groups of mice (5 mice per cage) were housed in 15x21x29 cm transparent cages bedded with wood shaving and equipped with a continuous flow of nipple watering devices. They were fed with pellets (Mice Pellets UNGA feeds) and tap water *ad libitum* throughout the experiment. The wood shavings in the cages were changed twice a week. The animals were handled as humanely as possible and in the same manner as before the onset of the experiment (they were not deprived of feeds and water). All the mice survived throughout the experiment. At the end of the experiment, the animals were immediately euthanized in a CO<sub>2</sub> chamber and incinerated.

### Plant material

The seeds were collected from the locally produced papaya (Kiim mountain) bought from Juja, Kalimoni area in Kiambu County, Kenya October, 2016 with the help of a botanist and a voucher sample collected for future reference. Seeds from ripe papaya fruits were used during this experiment.

### Preparation of the plant extract

#### Organic extraction

Mature healthy papaya ripe fruits were cut longitudinally using a knife and all the seeds were removed. The seeds were washed and left to dry. The samples were ground and exactly 50 g of the sample was weighed using a top balance and put in a flat-bottomed flask. Methanol and Dichloromethane DCM at a ratio of 1:1 was then added until the sample was completely immersed. The mixture was then agitated by shaking and then filtered using Butcher funnel: Whatman No 1 filter paper. The residue was re-extracted by adding the solvent and left to extract for 24 h. The extract was then concentrated to obtain a semi liquid mass using a rotary evaporator (0.1 bar) in water bath at 40°C.

#### Aqueous extraction

Distilled water was used for aqueous extraction. Mature healthy papaya ripe fruits were cut longitudinally using a knife and all the

seeds were removed. The seeds were washed and left to dry. The samples were ground and exactly 50 g of the sample was weighed using a top balance; it was put in a flat-bottomed flask and then mixed with distilled water. The extracts were then filtered using a Whatman paper and the filtrate was concentrated using a freeze drier to obtain the crude extracts

### Phytochemical screening

The seed extracts (methanol and aqueous) were screened for flavonoids, flavones, saponins, steroids, alkaloids, phenols and terpenoids using principled laboratory standard methods for each compound.

#### Flavonoids and flavones

1 g of extract was dissolved in 10 ml distilled water and then filtered using Whatman filter No.1. Magnesium turning of 10 mg was then added into 1 ml of the filtrate, followed by the addition of 0.05 ml concentrated sulphuric acid. The presence of magenta red indicated the presence of flavonoids (Brain and Turner, 1995).

#### Tannins

Half a gram (0.5 g) of the water extract was dissolved in 2 ml of distilled water and filtered. Two drops of ferric chloride were then added to the filtrate. A blue black precipitate indicated the presence of tannins (Harbone, 1998).

#### Saponins

The presence of saponins was determined by frothing test. Half a gram (0.5 g) of the plant extract was shaken in 5 ml of distilled water and allowed to stand for 10 min. No froth was formed

#### Steroids

Two milliliters of acetic anhydride was added to 0.5 g methanol extract of each sample with 2 ml H<sub>2</sub>SO<sub>4</sub>. The color changing from violet to blue/green indicated the presence of steroids (Brian and turner, 1995).

#### Alkaloids

Three drops of Mayer's reagent were added to 2 ml of the extract. Formation of a yellow colored precipitate indicated the presence of alkaloids.

#### Phenols

Three drops of ferric chloride solution were added to the extract. Formation of a blue black color indicated the presence of phenols.

#### Terpenoids

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of Terpenoids.

### Acute oral toxicity testing

Extracts at the dose range of 5, 50, 300 and 2000 mg/kg body

weight were administered using oral gavage to different groups of mice comprising 3 female mice in each group. On the day of the experiment, animals were starved of food for 2 h before drug administration. Animals were kept under close observation for 4 h after administering the fraction for behavior, neurological and autonomic profile and then observed for any change in the general behavior and/ or physical activities; any mortality was recorded within 72 h (OECD Guidelines, 2001).

### Hypoglycemic studies

#### Induction of experimental diabetes

Mice aged 6 weeks or older were fasted overnight and received a single injection of alloxan monohydrate (Alloxan, Sigma Aldrich, USA) at a dose of 150 mg/kg body weight intraperitoneally. The induction of alloxan-induced diabetes was confirmed by measuring blood glucose levels of each mouse after 7 days by following the blood glucose monitoring after alloxan administration. Mice with glucose levels above 150 mg/dl were considered diabetic and used for the efficacy study (Xu et al., 2008).

#### Blood glucose determination

Blood was obtained by a prick on the lateral tail vein and blood glucose determined using a glucometer based on the glucose oxidase method for normal and diabetic mice after an overnight fasting for the determination of the glycaemia (0 time). Treatments were administered orally and the blood samples approximately 0.5 ml per mouse were collected for blood glucose level estimation at specific time intervals. Daily treatment took place post-induction of diabetes and assessment of the glucose level was determined after 72, 96, 120 and 144 h post diabetes induction (Benedict, 1911).

### Statistical analysis

All data were analyzed and computed by SPSS and presented as mean  $\pm$  standard deviation (SD). A significant difference between control and experimental groups was assessed by the use of Student's t-test. Data from different treatments in the groups were compared using a one-way analysis of variance (ANOVA). Box plot was also generated to compare the groups. The level of significance was set at p values less than 0.05.

### Ethical considerations

This study was carried out at Kenya Medical Research Institute (KEMRI). Permission to carry out the study and ethical clearance was sought from KEMRI's Scientific and Ethics Review Unit (SERU) and KEMRI Animal Care and Use Committee (ACUC). The research was conducted in accordance with KEMRI guidelines on animal care and internationally accepted principles for laboratory animal use and care as found in WHO guidelines.

## RESULTS

### Acute oral toxicity studies

Table 1 shows the variation of weight measured in animals administered with methanol seeds extracts during the oral acute toxicity test. The weight of the mice

**Table 1.** Phytochemical analysis.

Test	Observation	Aqueous extract	Methanol extract
Saponins	No froth formation	-	-
Alkaloids	Yellow precipitate formed	+	+
Flavonoids	Magenta red color present	+	+
Tannins	Blue black precipitate formed	+	+
Phenols	Blue black color formed	+	+
Steroids	Color change: violet to blue green	+	+
Terpenoids	Brown coloration at the interface	+	+

Key: - Absent, + Present.

**Table 2.** Methanol extracts.

Groups	No. of animals	Weight at day 0(g)	Weight at day 7(g)	Weight at day 14(g)
Control	3	18.33 ± 0.577	21.67 ± 1.155	23.33 ± 1.154
5 Mg/Kg	3	20.00 ± 1.000	22.67 ± 1.528	26.00 ± 1.000
50 Mg/Kg	3	17.67 ± 0.577	21.33 ± 0.577	23.33 ± 0.577
300 Mg/Kg	3	18.00 ± 1.732	22.33 ± 1.528	23.67 ± 0.577
2000 Mg/Kg	3	18.33 ± 0.577	21.33 ± 0.577	22.67 ± 0.577

**Table 3.** Water extracts.

Groups	No. of animals	Weight at day 0(g)	Weight at day 7(g)	Weight at day 14(g)
Control	3	20.33 ± 1.155	25.00 ± 1.000	25.67 ± 0.577
5 Mg/Kg	3	21.33 ± 1.528	26.33 ± 1.528	27.33 ± 1.528
50 Mg/Kg	3	22.67 ± 1.528	26.67 ± 1.155	28.33 ± 1.528
300 Mg/Kg	3	21.33 ± 1.528	24.67 ± 1.155	26.00 ± 1.000
2000 Mg/Kg	3	24.67 ± 1.155	25.00 ± 1.732	29.00 ± 1.732

increased as the number of experimental days increased because of the regular uptake of food. Table 2 shows the variation of weight measured in animals administered with water seeds extracts during the oral acute toxicity test. The weight of the mice increased as the number of experimental days increased due to regular uptake of food, with the results almost similar to that of Table 3.

### Hypoglycemic studies

In Table 4 it was observed that the blood glucose concentration decreased as the number of days of experiment increased. Table 5 shows the *p* and sig. value. There was no statistically significant difference between the experimental crude *Carica papaya* seeds extract and the control drug which was metformin since the sig. value calculated was >0.05. Table 6 indicates the

summary of the analysis of variance between and within the experimental groups and the positive control group. There was no significant difference since the sig. >0.05 (Figure 1).

## DISCUSSION

### Phytochemical analysis

The methanol and water extracts from the seeds of *C. papaya* plant contain a number of phytochemicals such as flavonoids, phenols, steroids and tannins. All these compounds were present in both water and methanol extracts. The presence of flavonoids in the extracts indicates the naturally occurring phenolic compound with beneficial effects on the human diet such as antioxidant activity and neutralizing free radicals. Flavonoids present

**Table 4.** Blood glucose concentration.

Groups	Blood glucose concentration (mmol/dl)		
	Day 0	Day 5	Day 10
100 Mg/Kg water extract	19.325 ± 10.251	17.200 ± 6.784	17.993 ± 7.591
500 Mg/Kg water extract	24.920 ± 8.607	21.832 ± 7.009	21.728 ± 6.883
100 Mg/Kg methanol extract	12.620 ± 6.325	12.876 ± 6.816	12.725 ± 7.505
500 Mg/Kg methanol extract	15.940 ± 11.453	13.804 ± 8.477	10.831 ± 4.817
100 Mg/Kg metformin(positive control)	16.960 ± 11.925	15.936 ± 10.455	15.991 ± 10.476
Untreated mice	22.660 ± 10.127	20.688 ± 8.478	21.613 ± 7.922
Uninduced mice	6.825 ± 1.284	7.180 ± 0.700	7.069 ± 0.431

**Table 5.** Analysis of variation after the experiment.

Comparison between groups	Sig. level	Sig. value
100 Mg/Kg H <sub>2</sub> O vs. 100 Mg/Kg metformin	0.05	1.000
500 Mg/Kg H <sub>2</sub> O vs. 100 Mg/Kg metformin	0.05	0.873
100 Mg/Kg methanol vs. 100 Mg/Kg metformin	0.05	0.991
500 Mg/Kg methanol vs. 100 Mg/Kg metformin	0.05	0.937

**Table 6.** Annova.

Duration	F value	Sig. value
Day 1	1.063	0.402
Day 5	0.949	0.458
Day 10	1.402	0.273

a variety of biochemical and pharmacological actions that may affect the function of various mammalian cell systems. Several studies have shown that flavonoids, which are naturally occurring phenolic compounds and are widely distributed in plants, can act in lowering blood glucose in experimental models of diabetes. Such studies using isolated flavonoids like quercetin and myricetin, showed a reduction in the fasting blood glucose of diabetic mice induced by streptozotocin. (Goycheva et al., 2006)

Terpenoids and steroids were also present. Alkaloids have been used in medicines for reducing headaches and fever hence portraying antibacterial and analgesic properties. *C. papaya* seeds extract is a primary source of some of the secondary metabolites responsible for medicinal properties. This indicates that the plant can play a vital role in treating and managing some of the most severe diseases such as cancer, malaria, diabetes etc.

#### Acute oral toxicity

It was clearly observed that the mortality rate among the

groups after 72 h and even after 14 days for both methanol and water seed extracts was 0%. The mice maintained their normal activity viz being active, normal response to stimuli, normal appetite, and normal body temperature of 35°C. There was no isolation of any individual mice from the rest hence indicating no signs of stress and abdominal pain among the mice. The coat of the mice was also observed to be smooth and kempt. There was significant increase in weight from day 1 to day 14 with an average increase of 5.2 gms per mice in water and methanol seeds extract. This indicated that the water seed extracts had no effect on the uptake of food and the digestive system of the mice.

#### Hypoglycemic studies

During the hypoglycemic study, it was observed that there was a significant decrease change in the groups treated with both the extracts and the control drug, especially in the first five days. After that, there were still some significant changes observed but not as consistent as the first five days. The body weight of the animals increased significantly as the treatment continued with an

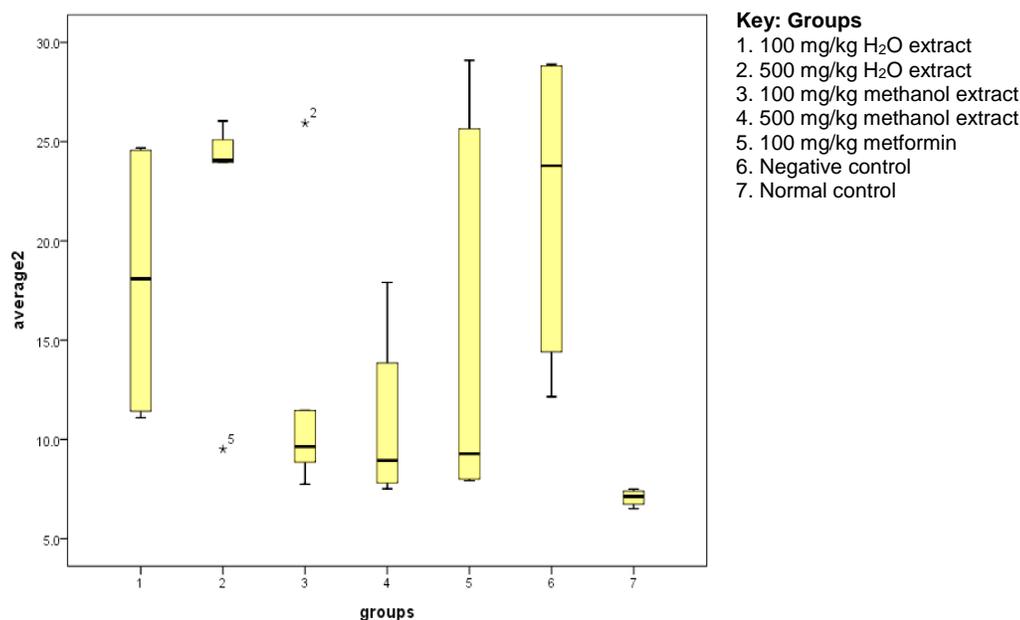


Figure 1. Box plot.

Table 7. Turkey's HSD results.

Groups	N	Subset for alpha=0.05
		1
4	5	10.825
3	5	12.720
5	5	16.000
1	5	18.000
2	5	21.740
Sig.		0.259

N- Number of animals per group (hypoglycemic studies was 5 mice per group, acute oral toxicity was 3 mice per group, OECD guidelines, 2001), Sig – significance value.

average of 6 gm for the ten days indicating that the treatment did not interfere with uptake of food or any alimentary activities of the animals. During the start of the experiment, all the seed extracts had no significant difference (sig. >0.05) as compared to metformin which was the positive control group; this indicates that they had effect the same way as the reference drug on the diabetic mice. Table 7 (Turkey's HSD results) indicated clearly that 500 mg/kg methanol extract dosage and metformin (positive control) 100 mg/kg dosage had the same mean variation of  $\leq 9$  mmol/dl on the blood glucose concentration; this indicated that they had the same effect in lowering the blood glucose concentration followed by 100 mg/kg methanol extract dosage, whose mean variation was  $\leq 10$  mmol/dl. 100 mg/kg H<sub>2</sub>O extract

dosage and 500 mg/kg

H<sub>2</sub>O extract dosage, even though had significant effect on lowering blood glucose concentration, presented the highest mean variation of all the groups that were treated. Negative control (untreated diabetic mice) also had a higher mean variation of blood glucose  $\geq 25$  mmol/dl since there was no treatment administered to them. Normal control (un-induced diabetic mice) recorded the lowest mean value of  $\leq 6$  mmol/dl, as they were not injected with alloxan and hence not diabetic.

## Conclusion

In conclusion, the crude extract of the *C. papaya* seeds

plays a role in the reduction of blood glucose through mechanisms that involve the reduction of hepatic glucose production and/or that promote the storage of glucose in the liver. The molecular mechanisms by which *C. papaya* seed extracts stimulate the hypoglycemic effects are still unknown. The characterization of the extracts of *C. papaya* seeds as hypoglycemic agent opens an interesting field of investigation requiring further studies about the mechanisms involved; this is because apart from hypoglycemic activities related to it, *C. papaya* seed extracts have been reported to have contraceptive effects on male mice among other pharmaceutical values.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Anaga AO, Onehi EV (2010): Antinociceptive and anti-inflammatory effects of the methanol seed extract of *Carica papaya* in mice and rats: African journal of pharmacy and pharmacology 4(4):140-144.
- Akah PA, Enwerem NM, Gamanuel K (2007): Preliminary studies on Purgative Effect of *Carica papaya* root Extract. Journal of fitoterapia 68:327-331.
- Aliyu BS (2006). Some Ethno medicinal Plants of savannah regions of West Africa description and phytochemicals: Triumph publishing company pp. 243-244.
- Aravind G, Debijit B, Duraivel S, Harish G (2013): Traditional and medicinal uses of *Carica papaya*. Journal of Medicinal Plants Studies 1(1):7-15
- Benedict SR (1911) The detection and estimation of glucose in urine. Journal of the American Medical Association 57(15):1193-1194.
- Brain KR, Turner TD (1995). Practical evaluation of phyto-pharmaceuticals. Wright-Scientifica Bristol 1:144-145.
- Burkill HM (1985): the useful plants of west tropical Africa: Royal botanical garden Kew 7(1):200-219.
- Chege MP (2010). Risk factors for type 2 diabetes mellitus among patients attending a rural Kenyan hospital. African Journal of Primary Health Care and Family Medicine 2(1):5.
- Chinoy NJ, Padman P (1996). Anti-infertility investigation on the benzene extract of *Carica papaya* seeds in male and female albino rats. Journal Aromatic Plant Science 18(3):489-494.
- Chinoy NJ, Trivedi D, Joshi H (1995). Effect of *Carica papaya* seed extract on female rat ovaries and uteri. Phytotherapy Research 9(3):169-175.
- Goycheva P, Gadjeva V, Popov B (2006). Mini-review Oxidative Stress And Its Complications In Diabetes. Trakia Journal of Sciences 4(1):1-8.
- Harborne A (1998). Phytochemical methods a guide to modern techniques of plant analysis: springer science and business media.
- International Diabetes Federation (IDF) (2011). Diabetes Atlas, 5th Edition. <http://www.idf.org/diabetesatlas/news/fifth-edition> release. Journal 15:323-325.
- Kirigia MJ, Hama BS, Luis GS, Saidou PB (2009). Economic burden of diabetes mellitus in the WHO African region BMC International Health and Human Rights 9:6
- Motala AA (2002) Diabetes trends in Africa. Diabetes/Metabolism Research and Reviews 18:S14-20.
- Parle M, Gurdita (2011). Basketful Benefits of Papaya: international research journal of pharmacy 2(7):6-12.
- Roberts KC, Shields M, de Groh M, Aziz A, Gilbert JA (2012). Overweight and obesity in children and adolescents: results from the 2009 to 2011 Canadian Health Measures Survey. Health reports 23(3):37-41.
- Sidi-Aliyu B (2006). Common Ethnomedicinal Plants of the Semiarid Regions of West Africa: Their Description and Phytochemicals (Vol. 1): Triumph publishing company.
- Yang L, Colditz GA (2015). Prevalence of overweight and obesity in the United States, 2007-2012. JAMA internal medicine 175(8):1412-1413.
- Xu Z, Wang X, Zhou M, Ma L, Deng Y, Zhang H, Zhao A, Zhang Y, Jia W (2008). The antidiabetic activity of total lignan from *Fructus Arctii* against alloxan-induced diabetes in mice and rats. Phytotherapy Research 22:97-101.

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