Asian Journal of medical Sciences 2(6): 266-270, 2010
ISSN: 2040-8773

Submitted date: December 06, 2010 Accepted date: December 22, 2010 Published date: December 24, 2010

Evaluation Analgesic, Anti-Inflammatory and Antiepileptic Effect of Hydro Alcoholic Peel Extract of *Punica granatum* (*pomegranate*)

[1]S. Olapour and [2]H. Najafzadeh
[1]Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran
[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

Abstract: *Punica granatum* L. is a widely used plant that has high nutritional value. The aim of present study was evaluation analgesic, anti-inflammatory and antiepileptic effect of Pomegranate Peel Extract (PPE) in mice. Hydro alcoholic peel extract of pomegranate was prepared by maceration method. In the first study the number of writhing was counted within 10 min in one group of mice after 10 min later to acetic acid injection intraperitoneally. In other group, the extract was intraperitoneally administrated at dose 400 mg/kg by 20 min before acetic acid injection and the number of writhing was counted as later group. In formalin test; the formalin was subcutaneously injected in foot of mice. The licking time of foot was calculated 5 and 15-25 min after formalin injection. In other group, test was done as previous but the extract was intraperitoneally administrated 400 mg/kg by 20 min before formalin injection. In the second study; the mice were divided in to 6 groups. One group of mice received strychnine at dose 3 mg/kg as negative control. Other group received Phenobarbital before strychnine administration as a positive control. In 4 groups of mice, the PPE was administrated at 100, 200, 400 and 600 mg/kg before strychnine administration. The onset, duration and number of convulsion were measured and time of death was determined in 30 min after strychnine administration. The results of first study showed that pomegranate peel extract was considerably decreased licking and writhing. The results of second study showed the PPE had significantly anticonvulsive effect. Thus, PPE has analgesic, anti-inflammatory and antiepileptic effect.

Key words: Analgesia, anti-inflammation, antiepilepsy, mice, pomegranate peel extract

INTRODUCTION

The pomegranate, *Punica granatum* L., is an ancient, mystical, unique fruit borne on a small, long-living tree cultivated throughout the Mediterranean region, as far north as the Himalayas, in Southeast Asia, and in California and Arizona in the United States. Pomegranate is a widely used plant that has high nutritional value (Poyrazog *et al*., 2002). In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. Numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, an ultraviolet radiation-induced skin damage (Parmar and Kar., 2007; Zhang *et al*., 2007). Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity (Hajimahmoodi *et al*., 2008; Nam *et al*., 2002).

Dell'agli *et al*. (2010) suggested about the beneficial effect of the fruit rind of *Punica granatum* for the treatment of malarial disease may be attributed to the anti-parasitic activity and the inhibition of the pro-inflammatory mechanisms involved in the onset of cerebral malaria (Dell'agli *et al*., 2010). The antimicrobial activity against some food-borne pathogens by various extracts from pomegranate fruit peels was evaluated using both in vitro (agar diffusion) and in situ (food) methods. The 80% methanolic extract of peels was a potent inhibitor for *Listeria monocytogenes*, *S. aureus*, *Escherichia coli* and *Yersinia enterocolitica* (Al-Zoreky *et al*., 2009).

The compounds of peel appear to be similar to fruit and flower of pomegranate especially punicic acid contain. Since the effect of PPE did not evaluate for anti-inflammatory, analgesic and anticonvulsant; aim of present study was evaluation analgesic, anti-inflammatory and antiepileptic effect of Pomegranate Peel Extract (PPE) in mice.

MATERIALS AND METHODS

This study was conducted in Iran-Ahvaz- Shahid Chamran University in 2009. After plant identification, the peels were shade dried and the powder yielded. The hydro-alcoholic extract was achieved by maceration

Corresponding Author: H. Najafzadeh, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz-Iran. Tel.: +986113330073/+989166182496

method in methane l70% from peel of pomegranate. The extract stored in refrigerator.

Mice were purchased from lab animals' research center, Jundishapour university of Ahvaz. Mice (NMRI strain) with 20±3 g weight were divided 6 mice in each group. The mice were kept with feed and water ad libitum under 12 h light and 12 h dark condition.

Acetic acid-induced abdominal writhing test: In the first study the number of writhing was counted within 10 min in one group of mice after 10 min later to acetic acid (1% solution) injection intraperitoneally. In other group, the extract at dose 400 mg/kg was intraperitoneally administrated 20 min before acetic acid injection and the number of writhing was counted as later group.

Formalin test: In formalin test the formalin was subcutaneously injected in foot of mice in another group. The licking time of foot was calculated 5 (acute phase) and 15-25 min (chronic phase) after formalin injection. In other group test was done as previous but the extract at dose 400 mg/kg was intraperitoneally administrated 20 min before formalin injection.

Anticonvulsive activity: In the second study the mice were divided in to 6 groups. One group of mice subcutaneously received strychnine at dose 3 mg/kg as negative control. Another group was received Phenobarbital at dose 20 mg/kg intraperitoneally before strychnine administration as positive control. In 4 groups of mice, the PPE was administrated at 100, 200, 400 and 600 mg/kg before strychnine administration. The onset, duration and number of convulsion were measured and time of death was determined in 30 min after strychnine administration.

Data analysis: The mean of data was compared between groups by SPSS software (version 16, USA) and p value was significantly concerned at 0.05 level.

RESULTS

The results showed the PPE extract decreased writhing in acetic acid test. The mean of number writhing in untreated group was 48.2 per 10 min. This mean significantly decreased to 10.8 per 10 min in treated group ($p<0.05$). The mean of licking time at acute and chronic phase in formalin test was 47.175 and 25.05 sec, respectively in control group. It significantly decreased to 3 sec in treated group ($p<0.05$). The PPE extract had analgesic effect in both acute and chronic phase.

The results of anticonvulsive activity of PPE showed the anticonvulsive parameters were better controlled at dose 200 mg/kg. The number of convulsions, starting of convulsion, time of convulsion and time of living in Fig. 1- 4 are illustrated. The anticonvulsive effect of PPE appears to be dose dependent.

DISCUSSION

At present study, we used acetic acid and formalin model for anti-inflammatory effect of PPE. The acetic acid induces inflammation in peritoan and causes writhing position. This model is not specific and 10% of animals usually do not shared in experiment and do not show writhing. But formalin test is more reliable than acetic acid test. The formalin model is widely used for evaluating the effects of analgesic compounds in laboratory animals. Injection of formalin into the hind paw induces a biphasic pain response; the first phase is thought to result from direct activation of primary afferent

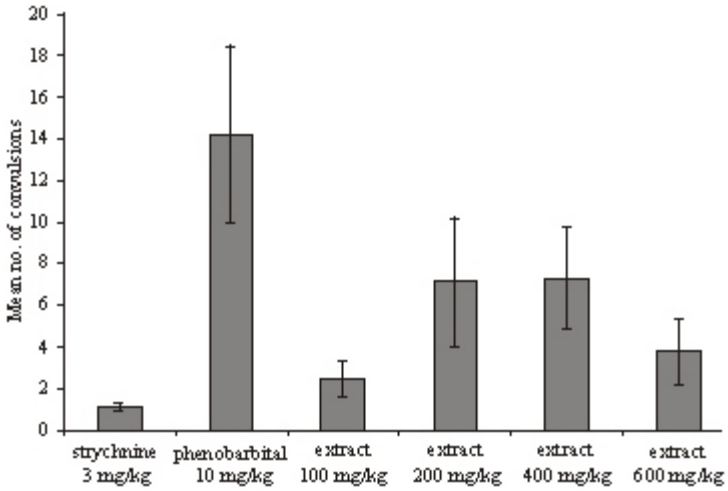

Fig. 1: Mean±S.E. of number of convulsions in different groups

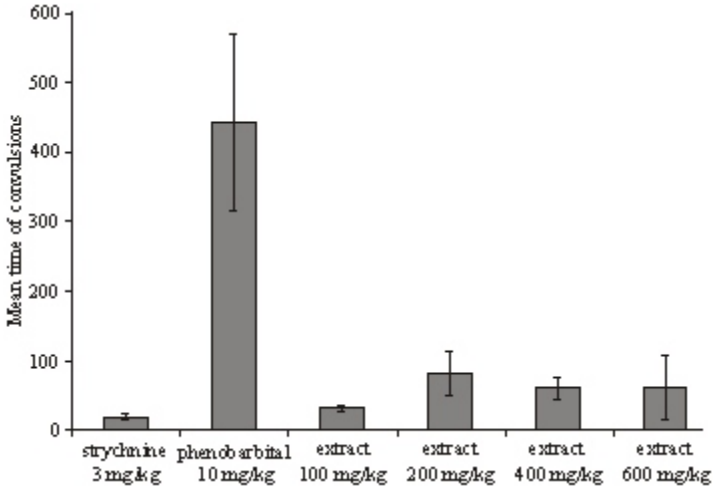

Fig. 2: Mean±S.E. of time of convulsions in different groups

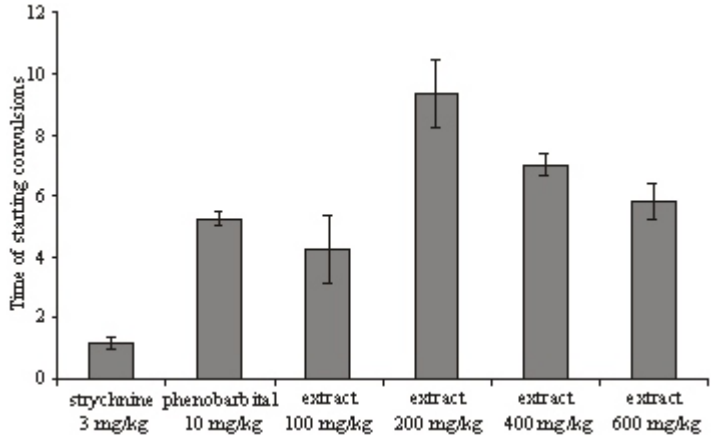

Fig. 3: Mean±S.E. of time of starting of convulsions in different groups

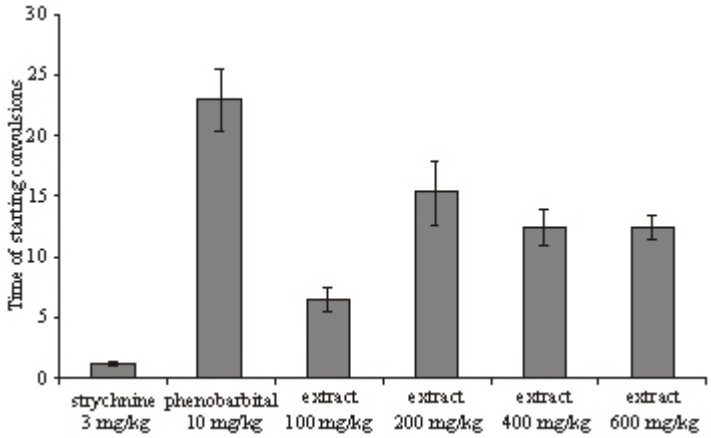

Fig. 4: Mean±S.E. of time of living in different groups

sensory neurons, whereas the second phase has been proposed to reflect the combined effects of afferent input and central sensitization in the dorsal horn (Colleen *et al*., 2007). There is need to study the therapeutic utility of *Punica granatum* as a valuable medicine to treat specific health problems. We demonstrated the PPE had anti-inflammatory and analgesic effect in animal models. Pomegranate extract may be used as a treatment in alternative medicine for inflammatory conditions, an anti-inflammatory effect by inhibiting the inflammatory cytokine-induced production of PGE2 and nitric oxide in vivo (Shukla *et al*., 2008). Haqqi (2008) showed that blood samples collected from rabbits fed pomegranate extract inhibited inflammation (Haqqi, 2008).

Punicic acid (PUA) caused a dose-dependent increase Peroxisome Proliferator-Activated Receptor (PPAR) alpha and gamma reporter activity in 3T3-L1 cells and bound although weakly to the Ligand Binding Domain (LBD) of human PPAR gamma. Dietary PUA suppressed NF-kappaB activation, TNF-alpha expression and upregulated PPAR alpha- and gamma-responsive genes in skeletal muscle and adipose tissue. Loss of PPAR gamma impaired the ability of dietary PUA to improve glucosehomeostasis and suppress inflammation (Hontecillas *et al*., 2009).

Toklu *et al*. (2009) showed that PPE supplementation reduced oxidative damage in the ileal tissues, probably by a mechanism that is associated with the decreased production of reactive oxygen metabolites and enhancement of antioxidant mechanisms. Adjuvant therapy of PPE may have a potential to support a successful radiotherapy by protecting against radiation-induced enteritis (Toklu *et al*., 2009).

Boussetta *et al*. (2009) showed that punicic acid exerts a potent anti-inflammatory effect through inhibition of TNFalpha-induced priming of NADPH oxidase by targeting the p38MAPKinase/Ser345-p47phox-axis and MPO release. They proposed the punicic acid can be used in inflammatory diseases such as inflammatory bowel diseases (Boussetta *et al*., 2009).

We demonstrated the PPE extract anti-convulsive effect on strychnine-induced seizures by dose dependent manner. This model was also used in other studies for anticonvulsive effect of herbal extract (Wahab *et al*., 2009). Patil *et al*. (2010) suggested that an orally administered aqueous root extract of *Ficus religiosa* has dose-dependent and potent anticonvulsant activities against strychnine- and pentylenetetrazole-induced seizures (Patil *et al*., 2010).

Strychnine is a competitive antagonist of the inhibitory neurotransmitter glycine at receptors in the spinal cord, brain stem and higher centers. It results in increased neuronal activity and excitability, leading to increased muscular activity (Wood *et al*., 2002).

CONCLUSION

In summary, pomegranate peel extract has analgesic and anti-inflammatory effect in formalin and acetic acid models and also has anticonvulsive effect in mice at strychnine model.

ACKNOWLEDGMENT

The authors would thank Dr. Aghel for her scientific helps.

REFERENCES

Al-Zoreky, N.S., 2009. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. Int. J. Food Microbiol., 15; 134(3): 244-248.

Boussetta, T., H. Raad, P. Lettéron, M.A. Gougerot-Pocidalo, J.C. Marie, F. Driss and J. El-Benna, 2009. Punicic acid a conjugated linolenic acid inhibits TNFalpha-induced neutrophil hyperactivation and protects from experimental colon inflammation in rats. PLoS One, 31; 4(7): e6458.

Colleen, R.M., J. Mandel-Brehm, D.M. Bautista, J. Siemens, K.L. Deranian, Z. Michael, J.H. Neil, A.C. Jayhong, J. David, M.M. Magdalene and M.F. Christopher, 2007. TRPA1 mediates formalin-induced pain. Natl. Acad. Sci. USA, 14; 104(33): 13525-13530.

Dell'agli, M., G.V. Galli, M. Bulgari, N. Basilico, S. Romeo, D. Bhattacharya, D. Taramelli and E. Bosisio, 2010. Ellagitannins of the fruit rind of pomegranate (*Punica granatum*) antagonize in vitro the host inflammatory response mechanisms involved in the onset of malaria. Malar J., 19(9): 208.

Haqqi, T., 2008. Anti-Inflammatory Effects of Pomegranate in Rabbits: A Potential Treatment in Humans? Drug Week, April, 07.

Hajimahmoodi, M., M.R. Oveisi, N. Sadeghi, B. Jannat, M. Hadjibabaie, E. Farahani, M.R. Akrami and R. Namdar, 2008. Antioxidant properties of peel and pulp hydro extract in ten Persian pomegranate cultivars. Pak. J. Biol. Sci., 11(12): 1600-1604.

Hontecillas, R., M. O'Shea, A. Einerhand, M. Diguardo and J. Bassaganya-Riera, 2009. Activation of PPAR gamma and alpha by punicic acid ameliorates glucose tolerance and suppresses obesity-related inflammation. J. Am. Coll. Nutr., 28(2): 184-195.

Nam, K., M. Rajendra, Y. Weiping, I. Neeman and T. Livney, 2002. Chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum* L.) for human breast cancer. Bre. Can. Res. Treat., 71: 203-217.

Parmar, H.S. and A. Kar, 2007. Antidiabetic potential of *Citrus sinensis* and *Punica granatum* peel extracts in alloxan treated male mice. Biofactors, 31(1): 17-24.

Patil, M.S., C.R. Patil, S.W. Patil and R.B. Jadhav, 2010. Anticonvulsant activity of aqueous root extract of *Ficus religiosa*. J. Ethnopharmacol., (In press).

Poyrazog, E., W. Knew and N. Atrik, 2002. Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. J Food Comp. Anal., 15: 567-75.

Shukla, M., K. Gupta, Z. Rasheed, K.A. Khan and T.M. Haqqi, 2008. Bioavailable constituents/ metabolites of pomegranate (*Punica granatum* L) preferentially inhibit COX2 activity ex vivo and IL-1beta-induced PGE2 production in human chondrocytes *in vitro*. J. Inflamm. (Lond), 13(5): 9.

Toklu, H.Z., O. Sehirli, H. Ozyurt, A.A. Mayadağli, E. Ekşioğlu-Demiralp, S. Cetinel, H. Sahin, B.C. Yeğen, D.M. Ulusoylu, V. Gökmen and G. Sener, 2009. Punica granatum peel extract protects against ionizing radiation-induced enteritis and leukocyte apoptosis in rats. J. Radiat. Res. (Tokyo), 50(4): 345-53.

Wahab, A., R. Ul-Haq, A. Ahmed, R.A. Khan and M. Raza, 2009. Anticonvulsant activities of nutmeg oil of Myristica fragrans. Phytother. Res., 23(2): 153-158.

Wood, D., E. Webster, D. Martinez, P. Dargan and A. Jones, 2002. Survival after deliberate strychnine self-poisoning, with toxicokinetic data. Crit. Care, 6: 456-459.

Zhang, Q., D. Jia and K. Yao, 2007. Antiliperoxidant activity of pomegranate peel extracts on lard. Nat. Prod. Res., 21(3): 211-216.