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Full Length Research Paper

Analgesic, anti GIT motility and toxicological activities of *Pistacia integerrima* Stewart ex Brandis bark in mice

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The study was carried out to screen the crude methanolic extract (Me. Ext) of *Pistacia integerrima* bark for anti gastrointestinal (GIT) motility and analgesic activities. Toxicological study was also conducted for safety profile of the bark. Balb-C mice of either sex weighing 25 to 35 g (n=6) were used as experimental animal. The Me. Ext was safe at a dose of 1000 mgkg^{-1} body weight (b.w), however at a dose of 1500 mgkg^{-1} b.w it caused 66.6% death of experimental mice. Me. Ext at a dose of 50 mgkg^{-1} (35.20±15.22% inhibition) and 100 mgkg^{-1} (38.89±9.28% inhibition) significantly (p<0.05) reduced the acetic acid induced writhing response in mice indicating significant antinociceptive effect compared to control group. The Extract at a dose of 100 mgkg^{-1} (28.93±6.98% inhibition) and atropine sulphate 2.5 mgkg^{-1} (42.93±0.47% inhibition) significantly (p<0.05) reduced the GIT motility, assessed by charcoal propulsion test. No significant difference was observed between control and Me. Ext at a dose of 50 mgkg^{-1}. These findings encourage the *P. integerrima* bark for its good pharmacological potentials. In addition the study also provides a scientific justifications to some of its tradititional uses.

Key words: *Pistacia integerrima* bark, methanolic extract, analgesic, anti GIT motility, toxicological effect.

INTRODUCTION

In spite of the overwhelming influences, our dependence on modern medicine and remarkable advances in synthetic drugs, a large segment of the world population still likes drugs from plants.

In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. Over three-quarters of the world population relies mainly on plants and plant extracts for primary health care (Premanathan et al., 2000). In the society of pakistan the traditional system of medicine has a deep roots. The traditional system of medicine is based on the use of medicinal plants, practiced by hakeems, unani healers, herbalists and traditional healers (Shinwari and Khan, 2000). Majority of the population in Pakistan, more than a half (66%) lives in rural areas and about 75-80% of them depend on traditional medicine system for basic health needs (Sheikh and Hatcher, 2005). So far a lot of medicinal plants were screened for toxicological, analgesic, and antidiarrheal activities (Qasheesh and Al-Rehaily, 2006; Turker and Camper, 2002). So keeping in view the long history and importance of medicinal plants the present research was undertaken.

Pistacia integerrima Stewart ex Brandis belongs to family Anacardiaceae. It is locally known as Shnaie in Pushto and kakar singhi in Hindi. The plant has been used in the traditional medicine for rheumatic fever, analgesic, antipyretic, antidiarrheal and hepatoprotective effects (Ahmad et al., 2008; Padulosi et al., 2002). The plant is a deciduous tree or shrub up to 30 m tall. The plant has single stem and possess multibranches, leaves are large up to 25 cm long and pinnate bearing 2 to 6 pairs of lanceolate and long leaflets. Roots are deep in the soil and large. Fruits are globular having diameter of 4 to 6 mm purplish and become blue on maturation (Padulosi et al., 2002; Flora of Pakistan, 1969). The phytochemical investigation of *P. integerrima* revealed

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that the leaves and bark of plant contain precious secondary metabolites like alkaloids, flavonoids, carotenoids, triterpenoids (Ismail et al., 2011; Ansari et al., 1993). The galls of the plant have also been screened for the presence of phytochemicals. The galls contain alkaloids, tannins, sterols, reducing sugars and flavonoids. Fatty ester, keto alcohol and triterpenic acid have been isolated from the galls of the plant (Ahmad et al., 2010). Tetracyclic triterpenoids from the galls have also been reported (Ansari et al., 1994). The antioxidant, xanthine oxidase inhibitory and anti gout activities of leaves have been reported (Ahmad et al., 2008, 2006). Antidepressant (Ansari et al., 1993), analgesic (Ansari and Ali, 1996), immunomodulatory (Joshi and Mishra, 2008), antioxidant and hepato protective (Joshi and Mishra, 2010) activities of *P. integerrima* galls have also been studied. The antimicrobial and phytotoxic activities of *P. integerrima* bark have been reported (Rahman et al., 2011). As the leaves and galls of the plant have good pharmacological potentials, having similar phytochemicals as the bark content. So the bark which is used traditionally must have some potential for pharmacological activities. Therefore the present research was carried out with a view to screen the bark of *P. integerrima* for some pharmacological activities.

MATERIALS AND METHODS

Pistacia integerrima bark

The plant material, that is, stem bark of *P. integerrima* was collected in the month of April 2010, from district Buner, Khyber Pukhtunkhwa. Prof. Dr. Muhammad Ibrar, Department of Botany, University of Peshawar carried out the identification and authenticity. A voucher specimen (No. 10420Bot) was kept in the Department of Botany, University of Peshawar for reference.

Preparation of methanolic extract (Me. Ext) of *Pistacia integerrima*

The stem bark of *P. integerrima* was dried under shade and grounded by electric grinder. The grinded powder was then extracted with methanol and concentrated by rotary evaporator to the semi solid form (37% yields). The crude Me. Ext was completely solubilized in normal saline (0.9% sodium chloride) and DMSO (1%) for use in the *in vivo* experiments.

Standard drugs used

Diclofenic sodium was obtained from Lowitt pharmaceutical Peshawar, Pakistan. Atropine sulphate was purchased from Sigma, USA.

Animals

Balb-C mice of either sex weighing 25 to 35 g were used as experimental animals. The animals were bred in the animal house, Department of Pharmacy, University of Peshawar. The animals were maintained in clean and hygienic conditions with optimum room temperature. Clean and properly dried food was given to the animals and water was changed on daily basis. The animals were handled for five to seven days prior to the start of experiment. Animals were divided into different groups comprising of six mice in each. The set of rules followed for animals experiment were approved by the local ethical committee (14/EC/Pharm).

Acute toxicity test

Acute toxicity of Methanolic extract (Me. Ext) was determined in Balb-C mice (n=6) of either sex weighing 20 to 30 g. Animals were fasted for 16 h before the start of experiment. Different doses of the extract were administered to each group. Control group were given equal volume of normal saline. The Animals were then allowed free access to food and water and were observed over a period of 24 h for mortality (Hosseinzadeh et al., 2000).

Analgesic effect

Acetic acid induced writhing test

Acetic acid induced writhing test was used for determination of analgesic effect. The analgesic effect was determined in Balb-C mice (n=6) of either sex, weighing 25 to 30 g. Food was withdrawn from animals 24 h before the start of experiment. Each group was given test doses and after 30 min 1% Acetic acid 10 $mlkg^{-1}$ body weight intraperitonialy (i.p). Control group was given equal volume of normal saline. Diclofenic sodium 20 $mgkg^{-1}$ body weight (i.p) was used as standard drug (Subhan et al., 2010; Qadrie et al., 2009).

GIT effect

Charcoal meal test

Charcoal meal test was used for determination of anti GIT motility of crude Me. Ext. Balb-C mice (n=6) of either sex, weighing 25 to 30 g were used. Animals were fasted for 18 h prior to experiment but allowed free access to water. Each animal was given test doses and standard group were given atropine sulphate 2.5 $mgkg^{-1}$ body weight. After 25 min of the administration of test doses, 1 ml of 10% charcoal meal suspension in 5% powder gum acacia was administered to each animal. Animals were then killed by cervical dislocation after 15 min of charcoal meal administration. Abdomen was opened and small intestine was dissected out. The total distance traveled by charcoal meal was determined and % GIT transit was calculated (Abere et al., 2010).

Statistical analysis

The statistical analysis of experimental data was performed by using GraphPad Prism 5, ANOVA followed by Dunnett's Multiple Comparison test. Value of $p<0.05$ were consider significant.

RESULTS AND DISCUSSION

The percent cumulative death of experimental mice, subjected to crude Me. Ext of *P. integerrima* bark at a dose of 500, 1000 and 1500 mg kg^{-1} for acute toxicological test were shown in Figure 1. The result indicated that crude Me. Ext was safe at a dose of 1000 $mgkg^{-1}$ body weight when administered. However when

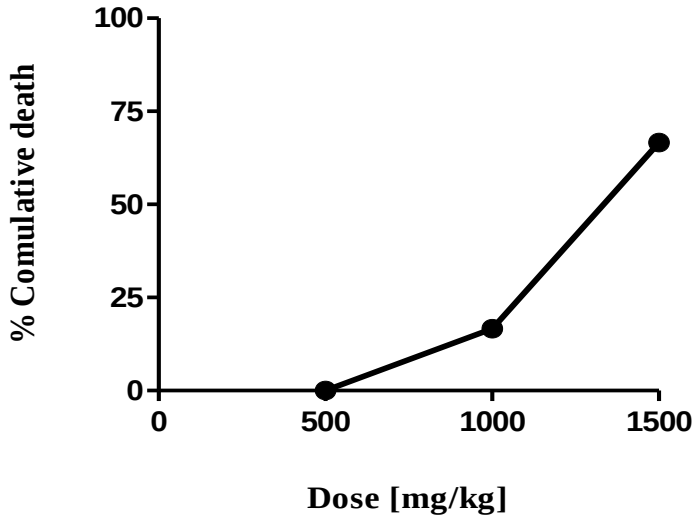

Figure 1. Dose dependent mortality in mice.

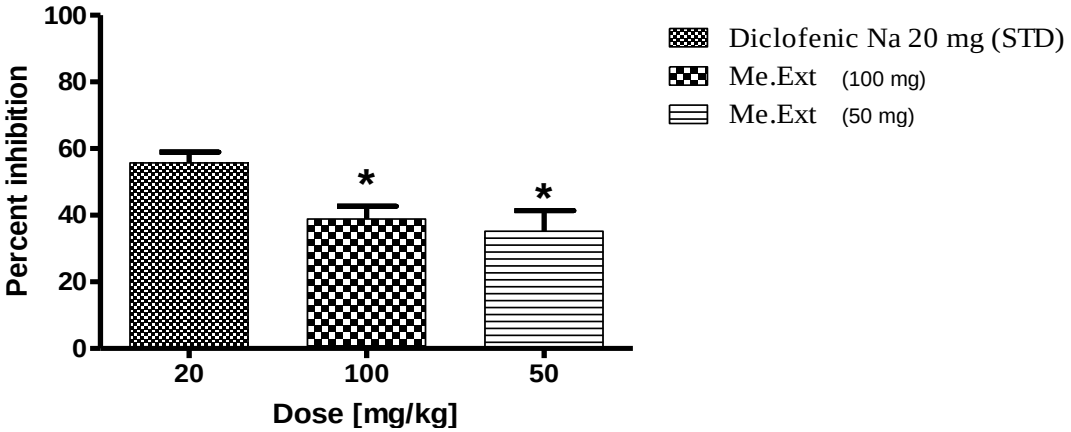

Figure 2. Percent inhibition of acetic acid induced writhes after different treatments. Each column represents Mean ± SEM (n=6). $p<0.05$, ANOVA followed by Dunnett's Multiple Comparison test revealed that Me. Ext at a dose of 50 mg and 100 mgkg^{-1} significantly reduced the writhing response in mice.

administered at a dose of 1500 mgkg^{-1} it caused 66.6% deaths of the experimental mice, therefore at a dose of 1500 mgkg^{-1} it was considerd to be toxic. The result also indicated greater therapeutic index for the Me. Ext of *P. integerrima* bark. The therapeutic index of a drug has an indirect relation with the toxicity of drug. Greater the therapeutic index safer will be the drug and vice versa. It has been reported that saponins causes hemolysis of red blood cells (Baumann et al., 2000). As the preliminary phytochemical study of Me. Ext of bark revealed the presence of saponins (Ismail et al., 2011), therefore at high concentration the toxic effect of *P. integerrima* may be due to the presence of high quantity of saponins.

The percent inhibition of acetic acid induced writhes in mice of crude Me. Ext of *P. integerrima* bark and standard drug diclofenic sodium were shown in Figure 2. The result showed that the crude Me. Ext of *P. integerrima* bark exhibited significant ($p<0.05$)dose dependent inhibition of acitic acid induced writhing in mice. Compare to diclofenic sodium (20 mgkg^{-1}) 73.4 % inhibition, the crude Me. Ext (100 mgkg^{-1}) 60.2% and (50 mgkg^{-1}) 50.22% inhibition was significant effect.

The results of Figure 2 indicated that the bark of *P. integerrima* possess analgesic potentials. The analgesic effect may be achieved through peripheral analgesic mechanism (through inhibition of cyclooxygenase, lipooxygenase and other mediaters or inhibition of pain responses mediated by nociceptors periphrally) or central analgesic mechanism (through inhibition of central pain receptors). The acetic acid induced writhing has been associated with increased level of PGE_2 and $PGE_{2\alpha}$ in the peritonial fluids. The increase in prostaglandienes levels

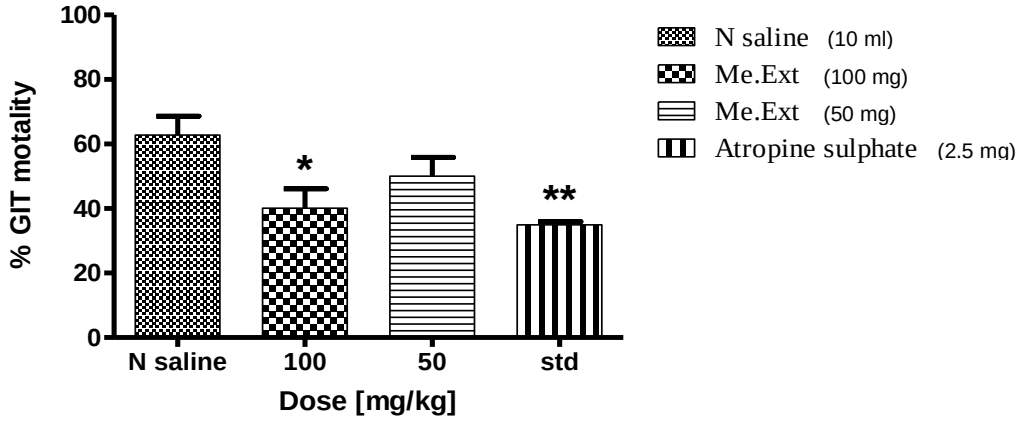

Figure 3. Percent GIT motility in mice after different treatments. Each column represents Mean ± SEM (n=6). $p<0.05$, ANOVA followed by Dunnett's Multiple Comparison test revealed that Me. Ext at a dose of 100 mgkg^{-1} and atropine sulphate 2.5 mgkg^{-1} b.w significantly reduced GIT motility. The test also revealed no significant difference between saline and Me. Ext at a dose of 50 mgkg^{-1}.

within the peritonial cavity then enhances inflammtory pain by increasing capillary permeability (Zakaria et al., 2008). Thus, the acetic acid induced writhing method was found effective to evaluate periphrally active analgesics (Zulfiker et al., 2010). Therefore, it is suggested that the bark of *P. integerrima* showing its analgesic effect through these mechanism. As the therapeutic efficacy of traditional treatment are often attributed through a combination of biologically active constituents which are the secondary metabolites of plants (Chindo et al., 2003), like *Strophanthus sarmentosus* (apocynaceae) contains secondary metabolites such as flavonoids, glycosides, tannins, alkaloids, saponins and account for therapeutic activities of the plant like analgesic, anti-inflammatory and antipyretic (Esther and Oluwole, 2011).

Some studies reported that, flavonoids are known to target prostaglandins (PGs) involved in acute inflammation and pain perception (Rajnarayan et al., 2001) and flavonoids have therefore been regarded with analgesic, anti-inflammatory and antipyretic activities (Mutalik et al., 2003). Since the preliminary phytochemical tests of *P. integerrima* bark revealed the presence of flavonoids (Ismail et al., 2011), so therefore it is concluded that the analgesic effect observed may be due to the presence of flavonoids.

The anti GIT tract motility effect of *P. integerrima* bark was shown in Figure 3. The results indicated that the crude Me. Ext at a dose of 100 mgkg^{-1} exhibited strong anti GIT motility effect assessed by charcoal propulsion method, comparable to that of muscarinic receptor blocking drug atropine sulphate. Blockage of muscarinic receptors in the GIT has dramatic effects on the GIT smooth muscle motility. Generally, tone and propulsive are diminished and increase intestinal transit time (Bertram, 2007). Therefore, it is suggested that the anti GIT motility of crude Me. Ext of *P. integerrima* bark may be due to the blockage of muscarinic receptors in the GIT tract. Since earlier studies reported that antidysenteric and antidiarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, and sterols (Galvez et al., 1991; Loganga et al., 2000). Hence, tannins, sterols, alkaloids are present in the crude Me. Ext of *P. integerrima* bark (Ismail et al., 2011) therefore these phytoconstiuents may be responsible for anti GIT motility effect of the *P. integerrima* bark.

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