INTRODUCTION

Diarrhoea has long been recognized as one of the most important health problems in the developing countries. Diarrhoea is the passage of abnormal liquid or unformed stool at an increased frequency. Infectious agents, certain medications, plant and animal toxins, gastrointestinal disorders, and substances that increase gastrointestinal tract secretions may cause it. It can also be caused by the ingestion of poorly absorbable materials, or inflammatory and dysmotility problems of the gastrointestinal tract (Palombo, 2006; Meite et al., 2009). Secretary diarrhoea is the most dangerous symptom of gastrointestinal problems and is associated with excessive defecation and stools being of abnormally loose consistency. It thus becomes important to identify and evaluate commonly available natural drugs as an alternative to currently used anti-diarrhoeal drugs, which are not completely free from adverse effects (World Health Organization, 1996).

Although currently used drugs are important in the management of diarrhoea, they are still linked with adverse effects and contraindications. For instance, racicadotril and loperamide are used to treat secretory diarrhea but they produce bronchospasm, vomiting and fever. Moreover, some are contraindicated in children below 6 years of age (loperamide) and intestinal obstruction (Thankurta et al., 2007). From a long time ago, plant kingdom played an

Evaluation of *Punica granatum* Peels Extracts and its Phenolic, Alkaloid and Terpenoid Constituents Against Chemically Induced Diarrhoea in Rats

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Abstract | The present study was carried out to investigate the anti-diarrhoeal activity of aqueous extract of peel of *Punica granatum* fruit and crude phenols, alkaloids, terpenoid extract in vivo by inducing diarrhoea in rats by oral administration of castor oil, enteropooling and small intestinal transit models in rats. The weight and volume of intestinal fluid induced by castor oil were studied by enteropooling method. Standard drug diphenoxylate (5 mg/kg, orally) was significantly reduced fecal output and frequency of droppings whereas crude phenols, alkaloids and aqueous extract, (at the doses of 20, 40 and 400 mg/kg orally respectively) significantly (P<0.001) reduced the castor oil induced frequency and consistency of diarrhoea and enteropooling. The gastrointestinal transit rate was expressed as the percentage of the longest distance travelled by the charcoal by the total length of the small intestine. Crude phenols, alkaloids and aqueous extract at the doses of 20, 40 and 400 mg/kg significantly inhibited (P<0.001) the castor oil induced charcoal meal transit. The crude phenols, alkaloids and aqueous extract of *Punica granatum* peel showed marked reduction in the number of diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as well as a higher reduction in intestinal transit. Results obtained to establish the efficacy and substantiate the folklore claim as anti-diarrhoeal agent. Further studies are needed to understand the complete mechanism of anti-diarrhoeal action of *Punica ranatum*.

Keywords | Antidiarrhoeal activity, *Punica granatum*, Phenols, Alkaloid
important role as medicinal plants that provide a potential sources for treatment of antidiarrhea (Palombo, 2006).

Pomegranate (Punica granatum L.) has been used in the folk medicine of many cultures, especially in the Middle East (Gurib-Fakim, 2006). Edible parts of pomegranate fruit represent 52% of the total fruit weight (El-Nemr et al., 1990). The non edible part called pomegranate peel extract or husk extract is primarily composed of alkaloids and polyphenols, which composed from anthocyanidins, pellar-gonidin, ellagotannins, gallic acid, ellagic acid, pseudopelletierine and isopelletierine. (Zakir, 2005; Navindra et al., 2006; Arun and Singh, 2012). Extracts of the bark, leaves, immature fruit and fruit rind have been given to halt diarrhoea, dysentery and hemorrhages (Ghani, 2003; Agunu et al., 2011). It is necessary to establish scientific evidences for therapeutic use of such traditional medicinal plants, as it may potentially be useful source of new effective therapies in the drug development process. Hence, this study aimed at evaluating the traditional claim of antidiarrhoeal and antisceretory effect of aqueous extract of peel of P. granatum and its acute toxicity in rat model.

MATERIALS AND METHODS

Preparation of Crude Aqueous Extract
Aqueous extraction of the Punica granatum peels was carried out by using (boiled distilled water) which is considered as very effective in extracting the active ingredients of the plant according to method described by (Qnais et al., 2007). A quantity of 150 g plant peels powder was put inside the flask with 3000 ml of distilled water and boiled for 15min with progressive stirring. The solution obtained was filtered through a filter paper. After that, the extract was dried by using an electric oven at temperature 30-35°C. The dry extract was placed in an incubator under 38-40°C for complete dryness of the sample. The final extract was kept frozen at −20°C until use.

Extraction of Active Ingredient in Pomegranate Peels Alkaloids
The extract was prepared according to the method of Harborne (1984). A quantity of 100 g of plant powder was homogenized in electrical blender with 350 ml of (4:1) ethanol. Distilled water the sample was filtered through muslin and then through a filter paper in Boukner funnel, then acidified by drops of (2% sulphuric acid) until the pH level dropped between 1 and 2. The solution was re-extracted with chloroform 3 times in the separating funnel until we got two layers; the upper one was neglected and the lower one was used. 2–3 drops of concentrated ammonium hydroxide were added to this layer until the pH became between 9 and 10. Then the solution was again extracted in the separation funnel with (1:3) chloroform: methanol twice and once with chloroform alone. After that the solution was separated into two layers; the upper layer (solvent) was neglected and the lower layer was evaporated in a rotary evaporator at 40°C for (1-2) hours, then oven dried until it turned into powder and the powder was kept in the refrigerator until use.

Phenols
Phenols were extracted according to Ribereau-Gayon (1972) and Harborne (1984). A quantity of 200 g of plant powder was divided into 2 equal portions, one was mixed with 300 ml of D.W. and the other one was mixed with 300 ml of 1% Hydrochloric acid. Then samples were homogenized in electric shaker for 5 minutes. The two mixtures were transferred to boiled water bath for 30–40 minutes, then cooled and filtered through muslin cloth and centrifuged with speed of 3000 rpm for 10 minutes. The two supernatants were mixed. Equal quantity of N-propanol was added to the mixture prior to sodium chloride was added until the solution was separated into two layers. The lower layer extracted in separating funnel with Ethyl acetate, and concentrated by using rotary evaporator at 40°C for 1–2 hours. The upper layer was dried by rotary evaporator at 40°C for 1–2 hours. The dried material of both layers were mixed and dissolved in 5ml of 96% ethanol, then left in oven until it turned into powder and kept in refrigerator until use.

Terpenoid
Terpenoid were extracted according to the method of Harborne (1984). A quantity of 15 g of plant powder was successively extracted in a soxhlet extractor for 24 hours with 200 ml chloroform. The solvent was removed by rotary evaporator at 40°C. Then the extract, dried in the oven at 40°C until it turned into powder and kept in the refrigerator until use.

Animals
Two hundred and ten male rats (200 – 250) were obtained from the Medicine College Center / Al-Nahrain University. These animals were kept under suitable environmental conditions of 20-25°C in an air-conditioned room and light period of 12 hours daily. The animals were housed in plastic cages of dimensions 20 × 50 × 75 cm and had free access to water ad libitum. The animals were kept for at least 2 weeks for adaptation before beginning the experiment.

Experimental Design
Castor Oil-induced Diarrhoea
Thirty five rats were divided equally into seven groups and housed in seven cages containing five rats each. Rats in group A, which served as a positive control, received distal water only, in group B received 5mg/kg of diphenoxylate hydrochloride and those in groups C, D, E, and F received...
Acute toxicity study was carried out using the method of Lorke (1983). One hundred and twenty rats were divided equally into twenty four groups, five rats / group. Six groups administered orally with 1000, 1200, 1400, 1600, 1800, and 2000mg/kg for aqueous extract, six groups for Phenols, six groups for alkaloid and six groups for terpenoid crude peel extract of *Punica granatum* administered orally with 50,100,200,400, 600 and 800mg/Kg body weight for phenols, alkaloid and terpenoid crude extract respectively. The rats were observed for signs of adverse effects and death for 24 h after treatment.

### RESULTS

#### EXTRACTION OF *Punica granatum*

Extraction of plant peels with distilled water gave a deep brown color extract with plant powder yield percentage of 3.3%. The dry peels of *Punica granatum*, was extracted for detection of alkaloids, phenols and terpenoid. The yield of major compounds in each extracts was determined in Table 1.

#### Table 1: Compounds quantity yielded from plant expressed as %

| Plant                  | Type of extract | Yield % |
|------------------------|-----------------|---------|
| *Punica granatum* peel | Crude aqueous   | 3.3%    |
|                        | Alkaloids       | 12.5%   |
|                        | Phenols         | 25.4%   |
|                        | Terpenoids      | 5.2%    |

This result is almost similar to the results of Qnais et al. (2007) who found that the percentage recovery of aqueous extract was 2.5% from 150 g of peels powder. The near similarity in yield percentage may be attributed to the same solvent which was used in the present extraction.

Results in Table 1 showed that the yield of phenols and alkaloids obtained from *P. Granatum* dry was higher (25.4%, 12.5%) respectively than terpenoid extracts. This result is similar to obtained by Omulokoli et al. (1997) and Monica et al. (2013) they mentioned positive result for phenols and alkaloids presence in high level and referred noted differences between plant parts, alkaloids and flavonoids in peel and flower were high than seeds and leaves. Saponins were highly present in the peel, seeds and flower than leaves, tannin were highly present in peel, leaves and flower than seeds.

#### CASTOR OIL-INDUCED DIARRHOEA

In the hour following castor oil oral administration, control
Table 2: The effect of *Punica granatum* peel extract, crude phenols, alkaloids and terpenoids extract on castor oil-induced diarrhoea in Rats

| Groups                          | Numbers of belts in 10 hours Mean±S.E | Number of diarrhoeal belts in 10 hours Mean±S.E | Percent of Inhibition |
|---------------------------------|---------------------------------------|-----------------------------------------------|-----------------------|
| Group A: + ve control           | 18.6 ± 0.03 a                         | 17.4 ± 0.6 a                                  | -                     |
| Group B: Diphenoxylate Hydrochloride 5 mg/kg | 7.6 ± 0.05 b                         | 3.8 ± 0.8 c                                  | 78.1%                 |
| Group C: PG extract 400 mg/kg   | 9.8 ± 0.06 b                         | 2.1 ± 0.08 cd                                 | 87.93%                |
| Group D: Crude Phenols extract 20mg/Kg | 7.2 ± 0.02 b                         | 0.0 ± 0.0 d                                  | 100%                  |
| Group E: Crude Alkaloids extract 40mg/Kg | 9.2 ± 0.5 b                          | 0.0 ± 0.0 D                                  | 100%                  |
| Group F: Crude Terpenoids extract 50mg/Kg | 8.3 ±0.05 b                          | 5.7 ± 0.05 b                                 | 67.24%                |

Group rat n= 5: Small letters refer to significant differences (P<0.05) among groups in the columns.

Table 3: The effect of *Punica granatum* peel extract, crude phenols, alkaloids and terpenoids extract on castor oil-induced enteropooling in Rats

| Groups                          | Weight of intestinal content (g) | Volume of intestinal content (ml) |
|---------------------------------|----------------------------------|----------------------------------|
|                                | Mean ±S.E % of inhibition | Mean ±S.E % of inhibition |
| Group A: +ve control            | 2.45 ± 0.07 a                   | -                               |
| Group B: PG extract 400 mg/kg   | 0.7 ± 0.04 b                    | 71.42%                          |
| Group C: Crude Phenols extract 20mg/Kg | 0.26 ± 0.2 d                 | 89.3%                           |
| Group D: Crude Alkaloids extract 40mg/Kg | 0.3 ± 0.04 cd               | 87.7%                           |
| Group E: Crude Terpenoids extract 50mg/Kg | 0.93 ± 0.09 b              | 62.04%                          |

Group rat n= 5: Small letters refer to significant differences (P<0.05) among groups in the columns.

group exhibited copious diarrhoea in 100% of the rats. Oral administration of phenols and alkaloids crude extract at 20 and 40 mg/kg respectively, induced a significant (p<0.05) antidiarrhoeal effect with the highest inhibition (100%) against castor oil-induced diarrhoea for up to ten hours post-administration comparative with partial inhibition of diarrhoea *P. granatum* at dose 400 mg/kg, which recorded (87.93%). While the standard drug Diphenoxylate Hydrochloride exhibited 78.1% inhibition of defecation. Phenols and alkaloid crude extract showed considerable activity in prevention of diarrhoea, frequency of stooling (reduction in the number of wet stools and total stools), decrease in weight of wet stools and the general diarrhoea score including the hard, mild and copious diarrhoea (Table 2). Only in the first hour after castor oil administration terpenoid crude extract at 50 mg/kg has significant protection against induced diarrhoea and showed considerable activity in delaying in onset of diarrhoea. The phenols and alkaloid extract produced a significant (p<0.05) greater inhibitory effects on all the diarrhoea parameters investigated than the highest dose of the *Punica granatum* peel extract.

**ANTI-ENTEROPOOILING TEST**
Castor oil induced accumulation of water and electrolytes intraluminal. Crude phenols and alkaloid extract at doses of 20 and 40 mg/kg reduction in intestinal weight and volume. Crude phenols and alkaloid extract produce 89.3% and 87.7% inhibition of weight, intestinal content respectively and volume of intestinal content was also reduced 87.35% and 84.48% with significance (P<0.05) when the *Punica granatum* peel extract was compared compared to terpenoid at dose 400 and 50 mg/Kg respectively. Treatment with the crude phenols and alkaloid extract produced a significant and dose-dependent reduction in intestinal weight and volume of intestinal content. This may promote reabsorption of materials in the intestine due to decrease propulsion of material in the intestinal tract, and the extract might have exerted its antidiarrhoeal action by antisecretory mechanism. This result similar to obtained by Izzo et al. (1999) and Uchida et al. (2009).

**GASTROINTESTINAL MOTILITY**
Crude of phenols, alkaloid and terpenoid extract was tested on gastrointestinal motility by charcoal meal test. The percentage decrease in intestinal transit time caused by atropine sulphate, crude extract of phenols and alkaloid was 73.99%, 73.08% and 71.39% at a dose 1, 20 and 40 mg/kg respectively.

These values are nonsignificant between them but significant (p<0.05) when compared with control group and *Punica granatum* peel extract and terpenoid. The result of *Punica granatum* peel extract and terpenoid shown that at doses of 400 mg/kg and 50 mg/kg caused a decrease in intestinal transit time by 59.42% and 56.17% respectively compared to control as shown in Table 4.

**ACUTE TOXICITY TEST OF AQUEOUS PEELS EXTRACT OF Punica granatum**
In the acute toxicity studies of oral administration of the aqueous peel extract, phenols, alkaloids and terpenoid
crude extract of *Punica granatum* none of the animals showed behavioral, neurological or physical changes. In addition, no mortality was observed at the test dose. Thus, the median lethal dose (LD50) of the plant extract was found to be greater than 2000 mg/Kg.

**DISCUSSION**

Orally administered castor oil at a dose of 1 ml induced diarrhoea after one hour, the reason for that is castor oil is known for its laxative effects due to its active component, ricinoleic acid. Castor oil is characterized by a high content about 90% of the hydroxylated unsaturated fatty acid ricinoleic acid mainly responsible for diarrhoea production (Ramakrishna et al., 1994). Ricinoleic acid is released by lipases in the intestinal lumen after oral ingestion of castor oil, and higher amounts of ricinoleic acid are absorbed in the small intestine and increases the peristaltic activity of the small intestine as a result of permeability of Na+ and Cl− changed in the intestinal mucosa (Qnais et al., 2007). The result of the current study is in agreement with (McKeon et al., 1999; Palombo, 2006) who showed an induced diarrhoea after oral ingestion of castor oil.

Yoshio et al. (1999) and Oben et al. (2006) emphasize that ricinoleate could stimulat the secretion of endogenous prostaglandin like Prostaglandins E and F which are considered to be good diarrheogenic agents in experimental animals as well as in human beings. The active metabolite ricinoleic acid in castor oil induces diarrhoea due to changing the electrolyte permeability by increasing electrolytes secretion, of the intestinal membrane, decreasing electrolytes absorption, and deranging intestinal motility causing a decreased transit time in addition to increased luminal osmolarity (osmotic diarrhoea) through elevating biosynthesis and releasing of prostaglandin which causes diarrhoea similar to some pathophysiological conditions. This result confirms those of (Galvez et al., 1993; Besra et al., 2002; Agbor et al., 2004; Brijesh et al., 2009; Gutiérrez et al., 2013).

Other causes of diarrhoea due to castor oil are decreased fluid absorption by inhibiting intestinal Na, K ATPase activity and increased secretion in the small intestine and colon, and affected smooth muscle contractility in the intestine, as reported by (Phillips et al., 1965; Nell and Rumel, 1984). In addition to activation of adenylylcyclase or mucosal cAMP mediated active secretion, and stimulation of endogenous prostaglandins E and F which causes stomach cramp and diarrhehea. Recnetly, platelet-activating factor and nitric oxide was contributed to the diarrhoea effect of castor oil as described by (Capasso et al., 1992; Galvez et al., 1993; Capasso et al., 1994; Mascolo et al., 1996; Uchida et al., 2000). The crude of phenols, alkaloids and aqueous extract of *Punica granatum* peels was evaluated for anti-diarrhoeal activity with less side effect than the conventional drug. The crude of phenols, alkaloids and aqueous extract of *Punica granatum* peels extract shows anti-diarrhoeal activity by reducing gastrointestinal motility and intestinal fluid accumulation. The anti diarrhoeal activity of *Punica granatum* peels extract could be returned to several mechanisms like the extract may increase the reabsorption of water and NaCl by reducing intestinal motility or it may reduce mucosal secretion and prevent prostaglandin release from intestinal mucosa.

The main chemical constituents in *Punica granatum* peels extract responsible for antidiarrhoeal activity are tannins, phenols, alkaloids, flavonoids and terpenoid by increasing colonic water and electrolyte reabsorption and inhibiting intestinal motility. This was affirmed by Farthing et al. (2008) and Daswani et al. (2010) who stated that the presence of the active ingredients particularly phenols, alkaloids, and terpenoid in the plant extract gave high activity in the inhibition of diarrhoea cases and the decrease of peristaltic index and it was similar to diphenoxylate hydrochord. Moreover, giving the aqueous *Punica granatum* peels extract and the crude of phenols, crude alkaloids and crude terpenoid extracts made reduction of total number of wet feces in the test groups of the experiment and this decrease was dose dependent and this might be due to the extract and the active ingredient which worked through the reduction of prostaglandins secretion from intestinal mucosa.
Liberation of ricinoleic acid by castor oil results in irritation and inflammation of intestinal mucosa, which lead to the release of prostaglandins and stimulation of intestinal secretion this was evident in the concentration of phentole (20 mg/Kg) and alkaloid (40 mg/Kg) and aqueous extract of *Punica granatum* peel (400 mg/Kg) inhibitors of prostaglandins biosynthesis are therefore considered to inhibit and delay castor oil-induced diarrhea. These results are in agreement with Ramakrishna et al. (1994), Qnais et al. (2007) and Sorin et al. (2012) who confirmed delay castor oil-induced diarrhoea as a result to inhibition of prostaglandin biosynthesis.

Prostaglandins are associated with changes in the bowel that stimulate diarrhoea. A recent study shows that the laxative effect of ricinoleic acid present in castor oil is due to the induction of contraction of intestinal smooth muscle which is mediated by activation of EP3 receptors on intestinal smooth-muscle (Brijesh et al., 2009). The *P. granatum* peels extracts and active ingredient exhibited insignificant inhibitory activity against castor oil induced diarrhoea at all doses. The results were dose dependent and comparable with that of standard drug as diphenoxyle hydrochlorid.

The therapeutic effect of diphenoxyle hydrochlorid is believed to be due to its anti-motility and anti-secretory properties. The mechanisms of anti-secretory action of diphenoxyle hydrochlorid have been discussed with reference to opiate agonism, block of calcium channels, and inhibition of calmodulin (Suzuki et al., 2000). From our results, it is likely that *P. granatum* peels extracts may mediate its effects through similar mechanisms.

The extract treated animals showed significant inhibitory activity against castor-oil induced diarrhoea and PGE2 induced enteropooling in rats. It is widely reported that different antidiarrhoeal agents exert their effect through different mechanisms such as inhibiting secretion, decreasing motility, delaying intestinal transit, reducing intraluminal fluid accumulation or by enhancing water absorption (Chitime, 2004; Abu Mohammed et al., 2013; Njinga et al., 2013). The crude of phenols, alkaloids and extract of *P. granatum* peels extracts produced an insignificant increase or decrease in propulsive movement at the standard charcoal meal in the small intestine, respectively suggesting a weak spasmyolytic activity. This activity was reverse dose dependent. The result of this experiment could be due to two types of receptors which appeared to be involved in extracts action on gastrointestinal tract: high and low affinity receptor subtypes sensitive to low and high agonist concentrations, which induced contraction and relaxation of smooth muscles respectively as found by Rouf et al. (2007) and Jalilzadeh-Amin et al. (2012) that the effect of aqueous peel extract is dose depended. Therefore, probably *P. granatum* peels extract at low doses increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal and by their anticholinergic and antihista-minic effects. This agreed with De Urbina et al. (1990) and Brankovic et al. (2009). The plant extract inhibited normal gastric emptying; this effect may be linked to the reduction in gastrointestinal propulsion observed in the rats. (Izzo et al., 1999; Camilleri, 2004; Uchida et al., 2009) showed decrease in intestinal transit time by morphine and atropine is linked to delays in gastric emptying. This suggests that the plant may have morphine-like action in exerting its antidiarrhoeal activity. The antidiarrhoeal activity of aqueous plant extract and active ingredient by using different dose level through regulating the gastrointestinal tract, slow down transit in the intestine, reduce colon flow rates and consequently have any effect on colonic motility this result is in support of previous claims in respect of antidiarrhoeal herbs.

**CONCLUSION**

The present study also confirms the presence of phenols, alkaloids, terpenoid in *P. granatum* is potential therapeutic option in the effective management of diarrhoea, thus justifying its widespread use by the local population for these purposes. Flavonoids and alkaloids more effective than terpenoid for inhibiting release of autacoids and prostaglandins, thereby inhibit secretion induced by castor oil. The given antidiarrhoeal activity of the rind extract of *P. granatum* may be due to the presence of previously mentioned phytochemicals present. It may be concluded that the present study supports the traditional use of the rind or aqueous extract of *P. granatum* by traditional medical practitioners in the treatment of diarrhoea and associated disorders.

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**CONFLICT OF INTEREST**

No any conflict of interest.

**AUTHORS CONTRIBUTION**

Through the availability of the fruit of the pomegranate and its benefits in preventing many diseases, including diarrhoea, it came the idea of a researcher at extracting the active ingredients and study of the effect in preventing diarrhoea.
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