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# TOXIC EFFECTS OF ALCOHOLIC EXTRACT OF LEAVES AND SEEDS OF *RICINUS COMMUNIS* IN MICE

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**ABSTRACT**

This study involved collection of *Ricinus communis* leaves and seeds from Diyala, then prepared *R. communis* leaves by drying and grinding by electrical grinder and put in glass container for extraction which was carried out by Magnetic stirrer with methanol to extraction. Extract of *R. communis* form was pasty with dark green in color. While the *R. communis* seeds are collection and removed the cover of it, then squeezing by mechanical squeezing to remove the oil and take out only castor seed cake and extract by petroleum ether and drying with Sodium hydroxide (NaOH) that give powder white in color called bean cake extract. The pilot study was done to determine the range of dose which used in experiment by using two mice for each dose and each extract. The doses of leave extract were started with (100mg/kg) which was given to two mice orally by stomach tube the volume of dosing was (100 ml) the dosing was calculated according to the weight of mice, the doses (200 mg/kg), (400 mg/kg), (800 mg/kg), (1000 mg/kg), (1200 mg/kg), (1400 mg/kg) and (1600 mg/kg), the dose that kill the animal is (1250 mg/kg). While the doses of bean cake extract with methanol were started with (100 mg/kg) which given to two mice the volume of dosing was (100 ml) according to the weight of mice. The doses (200 mg/kg), (400 mg/kg), (1600 mg/kg), (3200 mg/kg), (4000 mg/kg), (5000 mg/kg) and (5200 mg/kg), the dose that kill the mice is (5200mg/kg). The doses of bean extract by petroleum ether were started with (10mg/kg) was calculated according to the weight of mice. The doses (10 mg/kg), (20 mg/kg), (40 mg/kg), (80 mg/kg), (100 mg/kg), (120 mg/kg), (140 mg/kg) and (160 mg/kg) and the dose that kill the mice is (150 mg/kg).

Keyword: *Ricinus Communis,* Toxicity, LD50

## Introduction

The *Ricinus communis*; which has different names like, castor oil herb or Palma(e) Christi or wonder, trees, it belong to, Euphorbiaceous family (Worbs *et al*., 2011, Shah *et al*., 2015). *Ricinus communis*, herb, may be, originates from, Africa and in ancient times the herb used in Egypt or Romans and Greeks; now, the herb grows, wildly in many tropical and, subtropical regions, around the, world (Weiss, 2000). Its seed is, the castor bean, which despite its, name is not a true bean. Castor plant is indigenous to the, south eastern Mediterranean, Basin, Eastern Africa, and India, but is widespread throughout, tropical regions, and widely grown, elsewhere as an ornamental plant (Muhammad *et al*., 2015). The castor oil and the chemical intermediate prepared are used in pharmacology and in the production of such industrial products, as protective coatings, paints, synthetic, textiles, plasticizers, jet engine, lubricants, hydraulic fluids, soaps and detergents, resins, waxes, cosmetic, anti–fungal products and a variety of valuable derived products (Damirchi, 2011). Castor oil broken down in the small intestine to recinolic acid which is very irritating to gut promptly increases peristalsis (Abbas and Jaafar, 2010). Poisoning occurs when animals, including humans, ingest broken castor beans or break the seed by chewing: intact seeds may pass through the digestive tract without releasing the toxin (soto *et al*., 2002). The ricin toxin is found in two units, A series that inhibit the protein synthesis, and B series, which are linked to the galactose ligand on the cell membrane surface of animal cell (Noy-Porat *et al*., 2016 and Selvaprakash, 2017). This type of toxin enters the intestinal region in a chewing or crushing manner leading to serious toxicity symptoms, especially in the gastrointestinal tract GIT (Thornton, 2013). The method of exposure, to castor bean has a significant role in the, effect of toxin, inhalation, of a small amount of ricin toxin leads to severe respiratory, damage or death, which LD50 described in an experiment on mice 3-10 mg/kg, when inhaled or injected, and 30 Mg / kg when, chewing or digestion of plant, castor seeds (Tao *et al*., 2015). Also mentioned, the presence of high, toxicity of castor, bean during, an experiment on mice for, several plants, showed their, toxicity by, digestion, and said exposure to the castor plant during, digestion is more, dangerous than inhalation or, injection, and digestion of approximately, five beads may be lethal dose with the observation that, some of this amount, is analyzed by the effect of gastric acid, in addition, many researchers found that ricin toxin is one of the deadly toxins used in biological, weapons, it has been used in wars after being extracted from castor beans and documented, as a deadly toxin by means of digestion, and inhalation (Sarheed *et al*., 2018).

The decoction of leaves, is a purgative, lactagogue and emmenagogue (Almeida, 2003). A poultice, of the leaves is applied, to boils and, swellings, The hot leaves are, applied over the, abdomen of children to relieve flatulence, in women the ,leaves promote ,menstrual flow, tender leaves cure pain, in bladder (Kapoor, 2005). Leaves are also, recommended to relieve headache,and joint pains (Joshi, 2000, Bhattacharjee 2008 ,Jena and Gupta ,2012).

## Material and Methods

**Materials:**

1. Chemicals: The materials and chemical solution were used in laboratory experiment were:-

**Table 1 :** laboratory material used in laboratory experiments.

| Number | Materials and chemical solution | Company | Origin |
|---|---|---|---|
| 1 | Methanol absolute 99.9% | General drug house (GDH) | Thailand |
| 2 | Petroleum ether | ROMIL | United kingdom |
| 3 | Sodium hydroxide (NaOH) | FERAC | Germany |

**2. Equipment:**

**Table 2 :** The laboratory instrument were used in laboratory experiments.

| Number | system | Company | Origin |
|---|---|---|---|
| 1 | Balance | KERN PLE | Germany |
| 2 | Blender | Kenowood | Japan |
| 3 | Desiccator | | |
| 4 | Distiller | Exillo | England |
| 5 | Magnatic stirrer | Vision | Korea |
| 6 | Mask and caps | Meheco | China |
| 7 | Sterile gloves | Dar Al Hilal trading | raq |
| 8 | Metallic canula and tube | Local breed | Iraq |

**Animals**

Sixty nine mice were used in this study were obtained from cancer search center in at Yarmuk hospital. Animals ranged between 8-10 weeks of age, and their weight ranged between 25-35 g. animals kept under suitable environmental conditions of 20-25°C in an air-conditioned room and a regimen of 14 L: 10 D , Animals were housed in plastic cages of dimensions 12×15×29 cm, feed and water were given *ad libitum*. Care was taken to avoid any unnecessary stress. The cages were cleaned once a week. The animals were kept for at least 1 weeks for adaptation before experiment start. The experiment of this study was conducted in the animal house of Department at Veterinary Medicine College of Baghdad University.

**Extraction methods:**

**1. Castor Seeds Extract Preparation by (petroleum ether):** An amount of Seeds of *Ricinus communis* were collected from different shrubs distributed in the province of Diyala and classified and certified in S.B.S.T.C. in Iraq as mentioned previously in literature review. Seeds are cleaned and washed with tap water and then dried in open air and kept in special container till use. The outer husk was removed manually in order to get the white pulp, the decorticated castor seeds were pressed with mechanical hydraulic press for primary castor oil take out, and the result was friable texture material, this material mixed by the blender with petroleum ether for complete defatting of castor oil, the mixture was filtered by filter paper and special cotton tissue to separate the cake from the castor oil and petroleum ether. The cake was dried using desiccator by utilizing (NaOH) and the final result fine powder kept in special container contain anti-moisture sac to avoid the moisture until use (Al-Tahan, 1990).

**2. Castor Seeds Extract Preparation by (methanol):** After extract of castor bean by petroleum ether the castor seeds cake become like powder. Then the pulverized plant material (100g) was extracted for 72 h in methanol (1000ml) by using the magnetic stirrer. The separated extracts were then the extract was filtered by filter paper named Ac0%. Finally extract dried at room temperature.

**3. Castor leaves Extract Preparation :** The dried leaves of *R. communis* were finely grinded using electrical grinder and stored in air tight containers for further use. The pulverized plant material (100g) was extracted for 72 h in methanol (1000ml) by using the magnetic stirrer, the separated extracts were filtered by filter paper named Ac 0%. Finally extract viscous liquid at room temperature.

**Acute toxicity study**

**1. The pilot study:** The experiment was started using several doses with different concentration of *Ricinus communis* leave and bean extract orally. Fifty two male and female mice (Albino,sepring Dawlly Bo.CL ) were used to the pilot study. The doses of leave methanol extract were started with (100mg/kg) which was given to two mice orally with stomach tube the volume of dosing was (100 ml) the dosing was calculated according to the weight of mice, the doses (200mg/kg), (400mg/kg), (800mg/kg), (1000 mg/kg), (1200 mg/kg), (1400 mg/kg) and (1600 mg/kg) and the dose that kill the animal is (1500 mg/kg). While the doses of bean cake methanol extract were started with (100 mg/kg) which given to two mice the volume of dosing was (100 ml) according to the weight of mice. The doses (200 mg/kg), (400 mg/kg), (1600 mg/kg), (3200 mg/kg), (4000 mg/kg), (5000 mg/kg) and (5200 mg/kg) the dose that kill the mice is (5200 mg/kg). The doses of bean extract by petroleum ether were started with (10 mg/kg) was calculated according to the weight of mice. The doses (10 mg/kg), (20 mg/kg), (40 mg/kg), (80 mg/kg), (100 mg/kg), (120 mg/kg), (140 mg/kg), (160 mg/kg) and the dose that kill the mice is (160 mg/kg).

**2. Median lethal dose ($LD_{50}$) :** Median lethal dose of extract was conducted to estimate the toxic potency using "up and down" method (Dixon, 1965). The ranges of toxic doses were estimated by primary (Pilot) study for the extract.

**3. "Up and Down" Method :** The improved estimates are available directly in table (3). The (O) for the survive or no reflex and (X) for dead or loss of righting reflex, according to the rule to calculate the LD50 (Dixon, 1965).

LD50 = (Final test level) + (Value from table) (Difference between dose levels).

LD50 = Xf + kd

Xf= final test level.

K = value from table.

d= Difference within dose levels

**Table 3 :** Value of K for estimating LD50 from Up and Down. If the table is entered from the foot, the sign of K is to be reversed (Dixon, 1965).

| N | Second Part of Series | k for Test Series Whose First Part is | | | | | Standard Error of $LD_{50}$ |
|---|---|---|---|---|---|---|---|
| | | O | OO | OOO | OOOO | | |
| 2 | X | −.500 | −.388 | −.378 | −.377 | O | .88σ |
| 3 | XO | .842 | .890 | .894 | .894 | OX | .76σ |
| | XX | −.178 | .000 | .026 | .028 | OO | |
| 4 | XOO | .299 | .314 | .315 | .315 | OXX | .67σ |
| | XOX | −.500 | −.439 | −.432 | −.432 | OXO | |
| | XXO | 1.000 | 1.122 | 1.139 | 1.140 | OOX | |
| | XXX | .194 | .449 | .500 | .506 | OOO | |
| 5 | XOOO | −.157 | −.154 | −.154 | −.154 | OXXX | .61σ |
| | XOOX | −.878 | −.861 | −.860 | −.860 | OXXO | |
| | XOXO | .701 | .737 | .741 | .741 | OXOX | |
| | XOXX | .084 | .169 | .181 | .182 | OXOO | |
| | XXOO | .305 | .372 | .380 | .381 | OOXX | |
| | XXOX | −.305 | −.169 | −.144 | −.142 | OOXO | |
| | XXXO | 1.288 | 1.500 | 1.544 | 1.549 | OOOX | |
| | XXXX | .555 | .897 | .985 | $1.000^{+1}$ | OOOO | |
| 6 | XOOOO | −.547 | −.547 | −.547 | −.547 | OXXXX | .56σ |
| | XOOOX | −1.250 | −1.247 | −1.246 | −1.246 | OXXXO | |
| | XOOXO | .372 | .380 | .381 | .381 | OXXOX | |
| | XOOXX | −.169 | −.144 | −.142 | −.142 | OXXOO | |
| | XOXOO | .022 | .039 | .040 | .040 | OXOXX | |
| | XOXOX | −.500 | −.458 | −.453 | −.453 | OXOXO | |
| | XOXXO | 1.169 | 1.237 | 1.247 | 1.248 | OXOOX | |
| | XOXXX | .611 | .732 | .756 | .758 | OXOOO | |
| | XXOOO | −.296 | −.266 | −.263 | −.263 | OOXXX | |
| | XXOOX | −.831 | −.763 | −.753 | −.752 | OOXXO | |
| | XXOXO | .831 | .935 | .952 | .954 | OOXOX | |
| | XXOXX | .296 | .463 | .500 | $.504^{+1}$ | OOXOO | |
| | XXXOO | .500 | .648 | .678 | .681 | OOOXX | |
| | XXXOX | −.043 | .187 | .244 | $.252^{+1}$ | OOOXO | |
| | XXXXO | 1.603 | 1.917 | 2.000 | $2.014^{+1}$ | OOOOX | |
| | XXXXX | .893 | 1.329 | 1.465 | $1.496^{+1}$ | OOOOO | |
| | | X | XX | XXX | XXXX | Second Part of Series | |
| | | −k for Series Whose First Part is | | | | | |

total number of trials reduced by one less than the number of like responses at the beginning of the series. The example in the introduction is $N = 6$. For a series as follows:

$$OOOXXOXO$$

we have $N' = 8$ and $N = 6$.

## Result

### 1. Extract result

The result of castor seeds extract by petroleum ether was dry, whitish – beige as in (figure 1)

**Fig. 1 :** Castor seeds extract by petroleum ether

The result of castor seeds extract by methanol was liquid, whitish or transparent color – beige as in (figure 2)

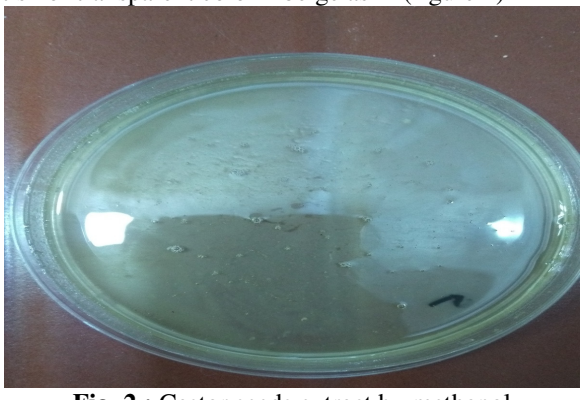

**Fig. 2 :** Castor seeds extract by methanol

The result of castor leaves extract by methanol was viscous liquid, green – beige as in (figure 3).

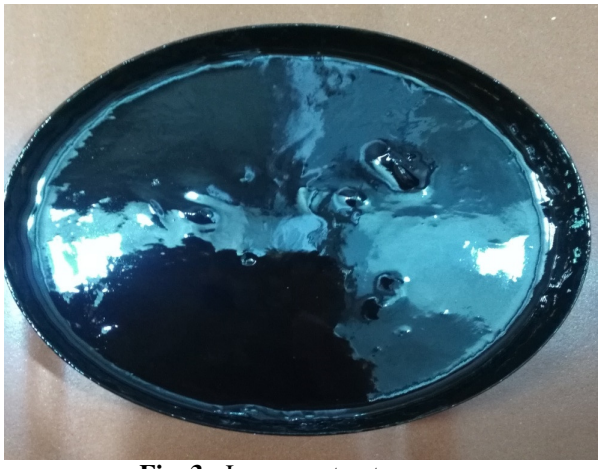

**Fig. 3 :** Leaves extract.

### 2- Pilot study result

In pilot study of castor leave extract by methanol the two mice was exposed to each dose ,the doses (100mg/kg) (200mg/kg), (400mg/kg), (800mg/kg), (1000mg/kg), (1200mg/kg), (1400mg/kg) and (1600mg/kg) and the dose when the animal is die is (1500mg/kg).( Table 4)

Table (4) Standard doses and dilution of leave extract of Ricinus communis for mice in pilot study

| Leave extract dose (mg/kg) | Solution concentration (mg/ml)* | No. of mice | No. of dead mice |
|---|---|---|---|
| 100 | 10 | 2 | 0 |
| 200 | 20 | 2 | 0 |
| 400 | 40 | 2 | 0 |
| 800 | 80 | 2 | 0 |
| 1000 | 100 | 2 | 0 |
| 1200 | 120 | 2 | 0 |
| 1400 | 140 | 2 | 0 |
| 1600 | 160 | 2 | 2 |

In pilot study of castor bean cake extract by methanol two mice was exposed to each dose, the doses (100 mg/kg), (200 mg/kg), (400 mg/kg), (1600 mg/kg), (3200 mg/kg), (4000 mg/kg), (5000 mg/kg) and (5200 mg/kg) the dose that kill the mice is (5200mg/kg). (Table 5)

**Table 5 :** Standard doses and dilution of bean cake extract by methanol of *Ricinus communis* for mice in pilot study.

| Bean cake extract Dose (mg/kg) | Solution concentration (mg/ml)* | No. of mice | No. of dead mice |
|---|---|---|---|
| 100 | 10 | 2 | 0 |
| 200 | 20 | 2 | 0 |
| 400 | 40 | 2 | 0 |
| 1600 | 160 | 2 | 0 |
| 3200 | 320 | 2 | 0 |
| 4000 | 400 | 2 | 0 |
| 5000 | 500 | 2 | 0 |
| 5200 | 520 | 2 | 1 |

In pilot study of castor bean cake extract by petroleum ether two mice was exposed to each dose, the doses (10 mg/kg), (20 mg/kg), (40 mg/kg), (80 mg/kg), (100 mg/kg), (120

mg/kg), (140 mg/kg), (160 mg/kg) and the dose that kill the mice is (160 mg/kg). (Table 6)

**Table 6 :** Standard doses and dilution of bean cake extract by petroleum ether of *Ricinus communis* for mice in pilot study.

| Bean cake extract Dose (mg/kg) | Solution concentration (mg/ml)* | No. of mice | No. of dead mice |
|---|---|---|---|
| 10 | 1 | 2 | 0 |
| 20 | 2 | 2 | 0 |
| 40 | 4 | 2 | 0 |
| 80 | 8 | 2 | 0 |
| 100 | 10 | 2 | 0 |
| 120 | 12 | 2 | 0 |
| 140 | 14 | 2 | 0 |
| 160 | 16 | 2 | 2 |

**3-Result of "Up and Down" Method**

The LD50 results of three type of extract were shown in table (7, 8 and 9)

So in the up and down method give the first animal 50 mg/kg b. wt ,the animal was live, so we give 2nd mice higher dose like 100 mg /kg b. wt. which also live, the 3rd mice was exposed to 150 mg / kg. b. wt., which also live, but the 4th mice was exposed to 200 mg / kg b. wt., which die, the 5th one exposed to 150 mg /kg. b. wt. remained live and the 6th mice exposed to 200 mg /kg b. wt. died. And the last animal was exposed to 150 mg /kg b. wt was lived. Formula: OOOXOXO The last dose was 150 mg, k factor from table for above formula is (0.741), the difference between doses=50

So LD50 of castor bean cake extract by petroleum ether through oral administration in mice: 150 + 50 (0.741) = 187.0 mg /kg b. wt.

**Table 7 :** $LD_{50}$ of bean cake extract by petroleum ether of Ricinus communis for (7) mice by "Up and Down" method.

| Bean cake extract Dose (mg/kg) | Solution concentration (mg/ml)* | Dead and survived mouse |
|---|---|---|
| 50 | 5 | O |
| 100 | 10 | O |
| 150 | 15 | O |
| 200 | 20 | X |
| 150 | 15 | O |
| 200 | 20 | X |
| 150 | 15 | O |

So in the up and down method give the first animal 4600 mg/kg b. wt ,the animal was live, so we give 2nd mice higher dose like 4800 mg /kg b. wt. which also live, the 3rd mice was exposed to 5000 mg / kg. b. wt., which also live, but the 4th mice was exposed to 5200 mg / kg b. wt., which die, the 5th one exposed to 5000 mg /kg. b. wt. remained live and the 6th mice exposed to 5200 mg /kg b. wt. died. And the last animal was exposed to 5000 mg /kg b. wt was lived. Formula: OOOXOXO The last dose was 5000 mg, k factor from table for above formula is (0.741), the difference between doses=200

So LD50 of castor bean cake extract by petroleum ether through oral administration in mice: 5000 + 200 (0.741) = 5,348.2 mg/kg. (Table 8)

**Table 8 :** LD50 of bean cake extract by methanol of *Ricinus communis* for (7) mice by "Up and Down" method.

| Bean cake extract Dose (mg/kg) | Solution concentration (mg/ml)* | Dead and survived mouse |
|---|---|---|
| 4600 | 460 | O |
| 4800 | 480 | O |
| 5000 | 500 | O |
| 5200 | 520 | X |
| 5000 | 500 | O |
| 5200 | 520 | X |
| 5000 | 500 | O |

So in up and down method we give the first animal 750 mg/kg b. wt, the animal was live, so we give 2nd mice higher dose like 1000 mg /kg b. wt. which also live, the 3rd mice was exposed to 1250 mg / kg. b. wt., which also live, but the 4th mice was exposed to 1500 mg / kg b. wt., which die, the 5th one exposed to 1250 mg /kg. b. wt. remained live and the 6th mice exposed to 1500 mg /kg b. wt. died. And the last animal was exposed to 1250 mg /kg b. wt was lived. Formula: OOOXOXO The last dose was 1250 mg, k factor from table for above formula is (0.741), the difference between doses=250

So LD50 of R. communis leaves extract through oral administration in mice: 1250 + 250 (0.741) = 1435.25 mg /kg b. wt. (Table 9)

**Table 9 :** LD50 of leaves extract by methanol of *Ricinus communis* for (7) mice by "Up and Down" method.

| leaves extract Dose (mg/kg) | Solution concentration (mg/ml)* | Dead and survived mouse |
|---|---|---|
| 750 | 75 | O |
| 1000 | 100 | O |
| 1250 | 125 | O |
| 1500 | 150 | X |
| 1250 | 125 | O |
| 1500 | 150 | X |
| 1250 | 125 | O |

## Discussion

The results obtained from this experiment were indicated to the toxic effect of the removed fat castor seeds and castor leaves extract by methanol, this effect can most probably attributed to the action of ricin and lectin (Sousa *et al*., 2017 and Vhutshilo, 2014), the extraction castor bean cake extracted with petroleum ether for 72 hours was white powder and from several other works like that of (Al-Khafaji *et al*., 2017). And the extraction of castor bean cake extracted by methanol for 72h was liquid whitish or transparent color. These results are identical to (AL-Jborrey *et al*., 2018). But castor leaves extract was greenish-black liquid after extract by methanol for 72h. these results are identical to (Anne *et al*., 2019). The LD50 of castor bean cake extract by petroleum ether was 187.0 mg /kg b. wt. and these results are not identical to (Al-Khafaji *et al*., 2017) that show the LD50 castor bean cake extract by petroleum ether is 352.58 mg. The LD50 of castor bean cake extract by methanol is 5348.2 mg/kg, these results are not identical to (AL-Jborrey *et al*., 2018) that show the LD50 of the castor bean cake extract by ethanol is 1100 mg/kg. And the LD50 of castor leaves extract by methanol is 1435.25 mg /kg b. wt. and these result are not identical to (Ihekuna *et al*., 2019) that show the LD50 of leaf

extract is above 2000 mg /kg b. wt. The toxic effect of castor bean cake that extract by petroleum ether is more than the toxic effect of castor bean cake that extract by methanol, It is may be that methanol removed some toxic substances from the seeds, including ricin, so its toxicity was less. and the toxic effect of castor leave extract less than castor bean cake that extract by petroleum .this due to different chemical composition between the leaves and bean , these result are identical to (Singh and Geetanjali, 2015) that show that Leaves the composed from: Aldehydes (C26 and C28), Alkanes (C26-C29), -Amyrin 1, -Amyrin 2, N-Butylmorpholine 3, Chlorogenic acid 4, Camphor 5, 1,8-Cineole 6, Citric acid23, -Caryophyllene 7, Decanamine20, N-Demethylricinine 8, Di-butylphthalate, 2,5-Dihydroxybenzoic acid (Gentisic acid), -Eleosteric acid 9, Ellagic acid 10, (-)-Epicathechin 11, Fumaric acid, Gallic acid 12 , Hexacosane-1,3-diol, 3-Hexen-1-ylacetate, Kaempferol 13, Kaempferol 3-O-D-glucopyranoside (Astragalin, 14), Kaempferol 3-O-D-xylopyranoside 15, Kaempferol 3-O-□-rutinoside (nicotoflorin, 16), Linoleic acid, Linolenic acid, Lupeol 17, Myristic acid 18, Malic acid, Methyl gallate, Neochlorogenic acid (an isomer of 4), 4-Octadecylmorpholine 19, Oleic acid, Palmitic acid 20, Palmitoleic acid, -Pinene 21, Primary alcohols (C22-C38), Quercetin 22, Hyperoside 23, Quercetin 3-O-Rutinoside (Rutin, 24), Quercetin-3-O-D-glucopyranoside (isoquercetin, 25)Quercetin 3-O-D-xylopyranoside (Reynoutrin, 26), Ricinine 27, □-Sitosterol 28, Stigmasterol 29, Stearic acid 30, Tartaric acid, Tannins. But the Seeds composition : Arachidic acid 34, Eicosenoic acid, Ergost-5-en-3-ol 35, Fucosterol, Glycine, Hydroproline, Leucine, Linoleic acid, Maltose, Oleic acid, Palmitinic acid, Phenylalanine39, Probucol, Proline, Ricinoleic acid (9Z,12R)-12-Hydroxyoctadec-9-enoic acid, 38), Stearic acid 30, Ricin, Ricinine 27, □-Sitosterol38, Stigmasterol 29, Tryptophan, Valine.

The different of doses of LD50 between different extract it may be due to method of extraction and this agreement with (AL-Jborrey *et al*., 2018), also the high doses of extract could be indicate nontoxic to animal and this identical with our present study.

## Conclusion

The LD50 of castor bean extract by methanol is (5348.2 mg/kg), and LD50 of castor bean extract by petroleum ether is (187mg/kg) and the LD50 of leaves extract is (1435.25mg/kg). The effect of castor bean extract by petroleum ether is more than leave extract and bean cake extract by methanol.

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