Phytochemical Screening and Hematological Effects of Bioactive Components of Aqueous and Methanolic Extracts of Leaves and Seeds of *Datura innoxia* on Male Wistar Rat

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Abstract

Datura is a genus belonging to family Solanaceae, consisting many species that include annual and perennial herbs, shrubs, and trees. The objective of the study was to determine the biochemical content of *Datura innoxia* seeds and leaves, in aqueous and methanolic extracts and to evaluate their toxic effects on experimental rats. The aqueous and methanol extracts were carried out using maceration method. The results of phytochemical screening of extracts revealed the presence of high concentration of alkaloids, flavonoids, tannins, steroids and triterpenes and low concentration of saponin and coummarins. 65 male Albino Wistar rats, three months old and with an average body weight ranged 110-120 g, were randomly divided into 13 groups, 5 rats for each. All groups received the same type of extract were administered 40, 60 and 80 mg/kg body weight, respectively. The effects of oral administration of leaves and seeds extracts to 60 healthy rats over 30 days were evaluated by histological studies, body weight changes, monitoring the chemicals disappeared in blood. The daily treated rats showed symptomatic signs of systematic toxicity such as shallow, convulsion and paralysis. The symptom at high dose (80 mg/kg), appeared immediately and persisted until death. The mortality percent of rats in 30 days after treatment orally with 40, 60 and 80 mg/kg of extracts was 0%, 75% and 100%, respectively. New compounds appeared in blood of treated groups. The histological results showed that administration of extracts caused pathologic changes in the liver, kidney and brain. The effects were less marked in the low doses. The treated groups had lower ($p \leq 0.05$) body weight gains than control group. *Datura innoxia* revealed the presence of important pharmacological bioactive substances.

Keywords: Phytochemical screening, Hematological, Extraction, *Datura innoxia*, Male Rat

1. Introduction

Datura is a genus belonging to family Solanaceae, consisting of 15 species that include annual and perennial herbs, shrubs, and trees. All the species are poisonous especially their seeds and flowers but used medicinally after detoxification by specific methods. *Datura* species are herbaceous, leafy annuals and short-lived perennials which can reach up to 2 m in height. *Datura* belongs to the classic "witches' weeds", along with deadly nightshade, henbane, and mandrake. Most parts of the plants are toxic, and *Datura* has a long history of use for causing delirious states and death. It was well known as an essential ingredient of potions and witches' brews. The genus name *Datura* is from the Bengali name 'dhatura'. *Datura* precise and natural distribution is uncertain, owing to its extensive cultivation and naturalization throughout the temperate and tropical regions of the globe. Today generally, experts classify only nine species of *Datura* (Preisseed, 2002). Domain: Eukaryota, Kingdom: Plantae, Order: Solanales, Family: Solanaceae, Genus: *Datura*, Species: *innoxia*. The *Datura* plant is spread widely all over the country and used traditionally without any care of toxicity. To establish the best and fast methods for *D. innoxia* toxic components analysis for cases which are usually received by forensic laboratories. The extracts of the plant are potentially poisonous and random use can cause restlessness and severe poisoning can lead to death. Since the earliest record of history the hallucinogenic properties of *Datura stramonium* are known (Biney and Nikoolov, 2006). Tropane alkaloids include atropine (dl-hyoscyamine) and scopolamine (i-hyoscine) (Firrdman, 2004). Atropine and scopolamine act as depressant of central nervous system and they affect the muscarinic cholinergic receptors. Many cases of suicide attempts by eating and chewing of *Datura* have been reported. It is very toxic plant because of the presence of toxic tropane alkaloids (Ekanem et al., 2016). There is a large database from poison information center's reporting *Datura* intoxications in children, teenagers and adults. Such data indicate that nearly all recent reports on exposure to *Datura* plant material are relating to abuse (due to the hallucinogenic effects) or experimentation with the plants (Torbu et al., 2002, Al shaikh and Sablay, 2005, Spina and Taddei, 2007, Montcriol et al., 2007, De Germond et al., 2008). In Taiwan, *Datura* intoxication was recorded in 14 people and caused by the ingestion of wild *Datura suaveolans* mistaken for edible vegetables (Chang et al., 1999). The herbal use of this plant has led to intoxications for example in Tunisia (Hamouda et al., 2000). Intoxications have been registered also after dietary exposure to other *Datura*

species, such as *Datura aurea* (Wilhelun et al., 2000), *Datura candida* (Greene et al., 1996). *Datura cornigera*, *Datura inoxia* (Pekdemir et al., 2004, Raman and Jacob, 2005).

2. Materials and Method

Plant samples (seeds and Leaves) collection and preparation

Datura innoxia (Figure 1: A, B and C) were collected from central El-Obeid City, North Kordofan State, Sudan, October, (2016). The plant leaves and seeds were cleaned, shade-dried and ground by a mechanical grinder. Extraction by petroleum ether and methanol and water was carried out according to method described by (Sukhdev et al., 2008).

Figure (1): Leaves and flowers of *Datura innoxia*.

Phytochemical Screening of Seeds and Leaves

The phytochemicals of the plant samples were estimated following the procedure adopted by screening for the active constituents was carried out using the methods described by (Harborne, 1998, Harborne, 1984, AOAC, 2000).

Animal Experimental Design

A total of 65 male Wistar albino rats divided into 13 groups, each group contain five rats; Group 1 served as control and fed with normal rat food and water for 30 days. Groups 2, 6 and 10 received aqueous seeds extract with doses of 40, 60 and 80mg/kg body weight/day respectively, While Groups 3, 7 and 11 received same types of aqueous leaves extract with doses of 40, 60 and 80mg/kg body weight /day respectively, Groups 4, 8 and 12 received methanol seeds extract with doses of 40, 60 and 80mg/kg body weigh/day respectively and Groups 5, 9 and 13 received methanol leaves extract with doses of 40, 60 and 80mg/kg body weight/day respectively. The extracts were administered to the rat's interagastrically using cathode tube. The method was prepared according to the Standard method of Organization for Economic Cooperation and Development (OECD 425). Daily feed intake is used to identify chemical compound in the blood of rats until the end of experiment. Clinical signs, average body weight and body weight gain is reported for each group. On day thirty, blood were collected for Histopathological study and chemical compounds

screening. Animal experiments were designed and conducted in accordance with the guidelines of institutional animal ethical committee.

Preparation of Rats' Whole Blood

Two ml of the blood sample was shaken well in a separatory funnel for 3 minutes then 2ml of distilled water was added to the blood sample and the mixture was shaken for 4 minutes to gain homogeneity.

Preparation of Basic Blood Extract

The pH of the blood sample adjusted to 10 by adding 200 μ l of 25% ammonium hydroxide and the mixture was shaken well by hand. 20ml of dichloromethane were added to the mixture in a separatory funnel and shaken for 3 minutes. Two layers appeared. The lower layer (dichloromethane layer) was drawn and filtered through a filter paper contained 10 g of anhydrous sodium sulphate.

Preparation of Acidic Blood Extract

The pH of the aqueous layer of the basic extract was adjusted to 4 by adding 0.5ml of dilute HCl, then 20ml of dichloromethane were added to the mixture in a separatory funnel and vortexed for 10sec, after a while two layers appeared. The lower layer (dichloromethane layer) was separated from the upper layer and filtered through a filter paper contained 10g of anhydrous sodium sulphate. The basic and acid bloods extracts were mixed together in a glass test tube and dried using nitrogen gas dryer. After the dryness 100 μ l of methanol was added and vortex for 10sec.

Histopathological study

Necropsy was conducted to identify gross lesion. The specimens of liver, kidney and brain were collected after slaughtering of rats and immediately fixed in 10% neutral buffered formalin (to prevent the post mortem changes) embedded in paraffin wax, sectioned at 5 μ m and stained routinely with hematoxylin and eosin using Harris's heamalun (Andrew et al., 2008).

Statistical Analysis

The data were analyzed by one- way analysis of variance using statistical package for social sciences (SPSS) 20th version followed by New – man Keul test (is a collection of statistical models and their associated estimation procedures, such as the "variation" among and between groups) used to analyze the differences between dose groups with control. All data were expressed as means $\pm$ standard deviations (M $\pm$ STD). P- values ($P \leq 0.05$) were considered statistically significant.

3. Results and Discussion

Table: 1 and 2 show the results indicated that the mean weekly body weight gain against daily oral doses of *D. innoxia* seeds and leaves aqueous and methanol extracts, for groups (2, 3, 4 and 5) at 40mg/kg/body weight, there was an increase of body weight, but had lower ($p \leq 0.05$) than control (group 1), while at 60mg/kg doses for groups (6, 7, 8 and 9) which received same extracts for 4 weeks, was an increase in body weight. Groups 10, 11, 12 and 13 which were fed with 80mg/kg dose per day they had more lowly mean than control. 80mg/kg dose per day of aqueous and methanol seeds and leaves extracts, they had more lower mean of body weight ($p \leq 0.05$) than group1(control) and groups (2, 3, 4, 5, 6, 7, 8 and 9).

In addition, it revealed that the methanol seeds and leaves extracts had a high toxicity than aqueous seeds and leaves extracts. Group 10 and group11 which were fed with 80mg/kg dose per day the mean of weekly body weight showed that they had most less ($p \leq 0.05$) mean of weekly body weight comparing to group (8 and 9) which received 60 mg/kg dose per day and 60mg/kg of methanol seeds and leaves extracts. Moreover, the mean weekly body weight of 80mg/kg of aqueous leaves was less ($p \leq 0.05$) than the same dose of aqueous seeds extract, Group 12 and group13 which were fed with 80mg/kg dose per day of methanol seeds and leaves extracts, the mean of weekly body weight showed that the most low ($p \leq 0.05$) mean of weekly body weight comparing with control. While the mean weekly of body weight for methanol seeds and leaves extract was lower ($p \leq 0.05$) than the methanol seeds at the same dose (80mg/kg). Regarding the control group, there is no significant difference in change of weight gains compared to other groups. These results agree with the results done by (Shama et al., 2014) in histopathological study of aqueous and methanolic extracts of *Datura innoxia* leaves and seeds on wistar rats and the results carried out by (Kalili and Atabis, 2004) in evaluation of analgesic effect of *Datura stramonium* leaves in hot plate and formalin test on male rats and the observation of body weight in groups (2, 3, 4 and 5) which treated with 40 mg/kg dose is agreement to the earlier report of (Crawford and Firedman, 1990) and who have noted no change in body weight of seeds *D. Stramonium* treated rats with the exception of an increase in the relative lung weight (Crawford and Firedman, 1990, Gidado et al., 2007). The possible mechanism could be that some of the β -cells might have survived the damage and secreted insulin when treated with seed extract. Toxicity studies of ethanol extract of the leaves of *D. stramonium* in rats (Crawford and Firedman, 1990).

Group 1 (control), rats were fed with normal rats' food and water. Group 2, 6 and 10 rats administered 40, 60 and 80mg/kg body weight aqueous seeds extracts respectively. Groups 4, 8 and 12 rats received 40, 60 and 80mg/kg methanol seeds extracts respectively. Group 3, 7 and 11 rats received 40, 60 and 80mg/kg body weight aqueous leaves extracts respectively. Group 5, 9 and 13 rats received 40, 60 and 80mg/kg body weight methanol leaves extracts respectively.

Table 1: Mean Weekly Body Weights of Rats Fed with Methanol and Aqueous Extracts of Datura Innoxia Leaves and Seeds for 4 Weeks.

Groups	0 time	1th week	2th week	3th week	4th week
G1	113.80 ±1.48	117.42±1.38	121.10±1.90	124.42±2.09	128.34±3.03
G2	112.60 ±2.07	114.14 ±2.36	116.76±2.04	118.96±0.99	121.36±1.87
G3	113.60±2.07	115.30±1.94	117.58±2.62	120.54±2.93	123.68±2.87
G4	113.60±2.07	115.38±2.61	117.04±2.53	119.14±2.97	121.32±4.08
G5	113.40±1.52	115.80±2.43	118.00±3.42	120.64±4.03	123.78±4.71
G6	113.80±2.39	113.74±3.06	114.45±4.22	116.56±5.75	116.83±6.79
G7	113.80±2.39	113.38±2.20	113.46±2.17	112.90±2.55	111.83±2.47
G8	113.80±2.77	113.20 ±2.71	112.68±2.19	111.92±2.22	111.30±2.32
G9	115.40 ±2.30	114.94±2.33	115.86±1.86	115.46±1.93	115.75±2.47
G10	114.20 ±2.77	113.08±2.71	113.96±1.66	114.15±0.21	113.50 ±0.00
G11	114.00±2.55	113.04±2.59	112.72±2.16	113.10±0.00	112.50±0.00
G12	113.80±2.77	112.98±2.66	113.27±2.80	112.60±0.00	0.00±0.00
G13	115.40±2.30	114.16±1.76	114.23±1.57	113.10±0.00	112.50 ±0.00

*Each value is average weight of 5 animals.

*Values in raw share same superscript letter show no significant.

Table 2: Comparative Significant Values of Body Weight:

Groups	Significant values	1 th week	2 th week	3 th week	4 th week week week
G1		0.111 ^{NS}	0.119 ^{NS}	0.011 [*]	0.024 [*]
G2		0.002 [*]	0.016 [*]	0.011 [*]	0.189 ^{NS}
G3		0.001 [*]	0.019 [*]	0.050 [*]	0.075 ^{NS}
G4		0.003 [*]	0.006 [*]	0.001 [*]	0.006 [*]
G5		0.007 [*]	0.014 [*]	0.020 [*]	0.040 [*]
G6		0.011 [*]	0.000 [*]	0.059	0.094 ^{NS}
G7		0.000 [*]	0.071 ^{NS}	0.074 ^{NS}	0.162 ^{NS}
G8		0.000 [*]	0.002 [*]	0.011 [*]	0.125 ^{NS}
G9		0.011 [*]	0.096 ^{NS}	0.122 ^{NS}	0.000 [*]
G10		0.000 [*]	0.139 ^{NS}	0.000 [*]	0.000 [*]
G11		0.000 [*]	0.011 [*]	0.000 [*]	0.000 [*]
G12		0.000 [*]	0.050 [*]	0.000 [*]	0.000 [*]
G13		0.002 [*]	0.011 [*]	0.000 [*]	0.000 [*]

*. The mean difference is significant at the $P \leq 0.05$ level. NS= (not significant).

The toxic symptoms at different dosages after oral administration of seeds and leaves extracts were given in tables 1 and 2. The clinical symptoms appeared on the rats due to the toxicity of active ingredients such as atropine, scopolamine and Hyoscyamine present in the extracts of *Datura innoxia* leaves and seeds especially which have anticholinergic effects. These results agreed with that obtained (Mohamed, 2007). In acute toxicity of aqueous and petroleum ether extracts of *Datura innoxia* leaves in mice (Shama, et al., 2014), in Biochemical and Histopathological study of aqueous and methanolic extracts of *Datura innoxia* leaves and seeds on wistar rats (Navaratanrojah, 2006), in Evaluation of analgesic effect of *Datura stramonium* leaves in hot plate and formalin test on male rats and study on *Datura metel* extract to Philids Ants (Navaratanrojah, et al., 2008).

Kidney section under the microscope of a rat treated with oral dose of methanol leaves extract for diffenents groups; showing congestion, oedema, dilatation of cells and renal tubules in the cortex, glomerular alteration (fatty cytoplasmic change) segmentation and packing in the cortex.

Brain sections under the microscopic tested of symptoms disease for the treated groups were shown in figures: (2) lows of Congestion and oedema of Brain cells and hemorrhage, which effected by the *Datura innoxia* leaves methanolic extract doses ,hyperemic vessels in cerebellum, cell degeneration, segmentation, In addition to that, hyperemic vessels in

cerebellum, inflammatory cell, infiltration in brain vessels, necrosis in brain tissue and degenerated neurons, and severe damages of tissue and cell.

Kedeny sections under the microscopic tested of symptoms disease for the treated groups were shown in figure: (3) showing oedema, dilatation of cells, renal tubules in the cortex and glomerular alteration, segmentation, kidney cells hemorrhage, nephritis, alteration, tubular nephrosis and cell degeneration. The lethal dose (LD50) of *Datura innoxia* seeds and leaves (methanol and aqueous) extracts in rats were nearly the same and parallel, that means they have the same efficacy due to their active ingredients this agreed with the results of acute toxicity of aqueous and petroleum ether extracts of *D. innoxia* leaves in mice (Mohammed et al., 2012).

Chemical Compounds of Rat's Blood Specimen (Control Group).

The chemical compounds of rats' blood of the control group determined using GC-MS were shown in table 3. About 19 compounds were detected in the blood sample. These compounds are as follows : Coummarins:, oxirane and Amides: (1-(2,4,6-Trisopropyl benzenesulfony) imidazole, Adipamide, Butanamide, Piperazine,2-methyl, fatty acids and esters: Benzeneaceticacid,4-(1,1-dimethylethyl)-,methyl ester, Triacontanoicacid, methyl ester, Hexadecanoic acid,14-methyl-,methyl ester, Eicosanoicacid, methyl ester, Tridecanoicacid, methyl ester, Heptadecanoic acid,16-methyl-,methyl ester, Docosanoicacid, methyl ester, Pentadecanoicacid, methyl ester, Acetic acid, chloro-

,hexadecyl ester, Phenols 2,6-di-tert-Butyl-4-(dimethylaminomethyl) Phenol, 2,4-Di-tert-butylphenol, Phenol,3,5-bis(1,1-dimethylethyl)-, Oxirane, {{4-(1,1-dimethyl ethyl) phenoxy}methyl}, ketones: (4ar-cis), 5,6-Dimethoxy-3-metyl-1-indanone, alkenes: 2(3H)- Naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,5-dimethyl ethylidene)-, Delta-Selinene, Quinones: (2,5-di-tert-Butyl-1,4-benzoquinone) and carboxylic compound(Benzene, 1,3-bis(1,1-dimethylethyl)-, 1,2-Benzenedicarboxylic).

Table 3: GC-MS Screening of Blood Specimen of Rats (Control Group).

Compound name	Formula	M. wt	Base ions	RT
2(3H)-Naphthalenone,4,4a,7,8hexahydro- methyl ester .dimethyl ethylidene)-, (4ar-cis).	C ₁₅ H ₂₂ O	218	161-218	7.611
1-(2,4,6-Trisopropyl Benzenesulfony) imidazole.	C ₁₈ H ₂₆ N ₂ O ₂	334	161-175	7.613
2,6-di-tert-Butyl-4-(dimethyl aminomethyl) phenol.	C ₁₇ H ₂₉ NO	263	203-161	7.612
Delta-Selinene	C ₁₅ H ₂₄	204	161-189	7.614
5,6- Dimethoxy-3-metyl- 1-indanone.	C ₁₂ H ₁₄ O ₃	206	191-206	7.82
Benzene, 1,3-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂	190	57-191	7.821
1,2-Benzenedicarboxylic	C ₂₀ H ₃₀ O ₄	334	149-121	10.82
2,5-di-tert-Butyl-1, 4- benzo quinone.	C ₁₄ H ₂₀ O ₂	220	205-177	11.35
Triacitanoicacid,methyl ester.	C ₃₁ H ₆₂ O ₂	466	74-143	11.581
Hexadecanoicacid, 14-methyl-, methyl ester.	C ₁₈ H ₃₆ O ₂	284	74-55	11.58
Eicosanoicacid, methyl ester.	C ₂₁ H ₄₂ O ₂	326	74-87	11.581
Tridecanoicacid, methyl ester.	C ₁₄ H ₂₈ O ₂	228	74-87	11.583
Heptadecanoic acid, 16-methyl- methyl ester.	C ₁₉ H ₃₈ O ₂	298	74-87	11.575
Docosanoicacid, methyl ester.	C ₂₃ H ₄₆ O ₂	354	74-87	11.58
Pentadecanoicacid, methyl ester.	C ₁₆ H ₃₂ O ₂	256	74-87	11.584
Acetic acid, chloro-, hexadecyl ester.	C ₁₈ H ₃₅ ClO ₂	318	97-83	12.36
Adipamide.	C ₆ H ₁₂ N ₂ O ₂	144	44-59	13.85
Butanamide.	C ₄ H ₉ NO	87	44-59	13.82
Piperazine, 2-methyl.	C ₅ H ₁₂ N ₂	100	44-57	11.08

Compounds of Rats' Blood Specimen Treated with 40mg/kg Aqueous Extract of Seeds.

The chemical compounds of rats' blood specimen supplemented with 40mg/kg with their retention time, molecular formula and molecular weight were shown in table 4. The GC-MS analysis showed 32 compounds. These compounds are as follows: Alkaloidial compounds (Scopolamine, Atropine, tropine, tropinone, 3-Tropanone, Pseudoecgonine methyl ester, Apotropan, Hyoscyamine, Tropacocaine, Tiglypseudotropin, Benzoylecgonine, Homatropine, Piperidine,1,1-methylenebis-, Pyrazine,methoxy-, 2H-Azepin-2-one,hexahydro-1-methyl and Tropic acid), amides and amino acids compounds (3-Nitrobenzylbromide, Creatine (Glycine,N-(aminoiminomethyl)-N-methyl), 1-(2,4,6-

Trisopropyl benzenesulfonylimidazole and 1H-Imidazole-1-propanamine, 4-Hydroxy-6-aminopyrimidine, fatty acids and esters (n-Butyric acid, 2-ethylhexylester, Linoleic acid ethyl ester, 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-, 9,12-Octadecadienoic acid (Z,Z)-, Erucic acid, Olic acid, Hexylisobutylcarbonate (carbonic acid), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, 9,12-Octadecadienoyl chloride, 9Z,Z)-, 6,9-Octadecadienoic acid, methyl ester, 9,11-Octadecadienoic acid, methyl ester, (E,E), 9,12-Octadecadienoic acid (z,z)-, methyl ester, hexylisobutylester, Di-n-octylphthalate (1,2-Benzenedicarboxylic acid, dioctyl ester), Butanoic acid, octylester, 3-Hydroxy-4-methoxybenzoic acid (Isovanillic acid), Uric acid, Vanillic acid). Presence of ketone compounds (Glacetophenone, 2(3H)-Benzofuranone, 3,3-dimethyl-5[(methylsulfonyl)oxy], Coummarin compounds (2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl), Terpenoidal compounds (Limonene oxide trans), Achols (2-(hydroxymethyl)-2-nitro (glycerol), Phytol, Carbohydrates (Sucrose), (Tryptophol), Quinone compounds (P-Benzoquinone, 2,6-di-tert-butyl-) and Phenols: (Phenol, 2,6-bis(1,1-dimethyl)-4-methyl- (Antioxidant), 2,4-Di-tert-butylphenol and Phenol, 2,5-bis(1,1-dimethylethyl).

Table 4: Compounds of Rats' Blood Specimen Administered with 40mg/kg Aqueous Extract of Seeds.

Compound Name.	Formula	M. wt	Base ions	RT
2(3H)-Benzofuranone ,3,3-dimethyl-5 [(methylsulfonyl) oxy].	C ₁₁ H ₁₂ O ₅ S	256	149-177	9.033
P-Benzoquinone, 2,6-di-tert-butyl.	C ₁₄ H ₂₀ O ₂	220	205-57	12.262
Phenol,2,6-bis(1,1-dimethyl)-4-methyl-.	C ₁₅ H ₂₄ O	220	205-57	12.274
9,11-Octadecadienoic acid,methyl ester,(E,E).	C ₁₉ H ₃₄ O ₂	294	95-81	13.851
9,12-Octadecadienoic acid(Z,Z)-	C ₁₈ H ₃₂ O ₂	280	95-81	13.850
9,12-Octadecadienoyl chloride,9,Z,Z)-.	C ₁₈ H ₃₁ ClO	298	81-95	13.854
6,9-Octadecadienoic acid,methyl ester.	C ₁₉ H ₃₄ O ₂	294	294-81	13.844
9,12-Octadecadienoic acid(z,z)-,methyl ester.	C ₁₉ H ₃₄ O ₂	294	294-81	13.823
9,12,15-Octadecatrienoic acid,methyl ester,(Z,Z,Z)-	C ₁₉ H ₃₂ O ₂	292	81-95	13.852
Piperidine,1,methylenebis-	C ₁₁ H ₂₂ N ₂	182	40-98	4.284
Pyrazine, methoxy-.	C ₅ H ₆ N ₂ O	110	110-81	6.454
Tropic acid.	C ₉ H ₁₀ O ₃	166	91-104	7.733
1H-Pyrrole, 1-butyl-.	C ₈ H ₁₃ N	123	81-123	6.451
4-Hydroxy-6-amino pyrimidine.	C ₄ H ₅ N ₃ O	111	111-83	7.902
Tryptophol.	C ₁₀ H ₁₁ NO	161	161-130	8.053
Phenol,2,5-bis(1,1-dimethyl ethyl)-.	C ₁₄ H ₂₂ O	206	191-206	8.262
2,4-Di-tert-butylphenol.	C ₁₄ H ₂₂ O	206	191-57	8.263
Scoparone. (Coumarin, 6,7-dimethoxy-).	C ₁₁ H ₁₀ O ₄	206	191-163	8.271
Linoleic acid ethyl ester.	C ₂₀ H ₃₆ O ₂	308	81-67	13.851
Olic acid.	C ₁₈ H ₃₄ O ₂	282	55-83	14.244
Erucic acid.	C ₂₂ H ₄₂ O ₂	338	69-55	14.241
Scopolamine	C ₁₇ H ₂₁ NO ₄	289	124-94	14.364
Hyoscyamine	C ₁₇ H ₂₃ NO ₃	289	124-82	13.261
Atropine	C ₁₇ H ₂₃ NO ₃	289	289-124	14.373
Apoatropin	C ₁₇ H ₂₁ NO ₂	271	124-82	14.372
Tropacocaine	C ₁₅ H ₁₉ NO ₂	245	140-124	14.374
Phytol.	C ₂₀ H ₄₀ O	296	44-111	15.063
Arginine.	C ₆ H ₁₄ N ₄ O ₂	174	44-69	15.511
1H-Imidazole- 1-propanamine.	C ₆ H ₁₁ N ₃	125	81-40	6.454
8,11,14-Eicosatrienoic acid, (Z,Z,Z)-.	C ₂₀ H ₃₄ O ₂	306	44-79	15.823
Di-n-octylphthalate (1,2-Benzenedi carboxylic acid, dioctyl ester).	C ₂₄ H ₃₈ O ₄	390	167-149	18.233
Acetophenone-2- carboxylic acid.	C ₉ H ₈ O ₃	164	149-40	9.034

Chemical Compounds of Rats' blood Specimen Treated with 40 mg/kg Aqueous Extract of Leaves.

The chemical compounds of rats' blood specimen treated with 40mg/kg aqueous extract of leaves and their molecular formula, molecular weight and retention time are shown in table 5. The GC-MS analysis revealed 19 compounds. These compounds are as follows: Alkaloidal compounds (Scopolamine, Atropine, tropine, tropinone, 3-Tropanone, Pseudoecgonine methyl ester, Apoatropin, Hyoscyamine, Tropacocaine,

Tiglypseudotropin, Benzoyl ecgonine, Homatropine and Tropic acid, Pyrrolidine,1-(2-methyl-1-propenyl), (3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyridinone, fatty acids and esters (2-methylpyrazine-5-carboxylic acid, Azelaic acid, Oleic acid, Acetic acid, phenyl (Benzeneacetic acid), Valeric acid, 5-Aminovaleric acid, 3-methyl-, 3-Methyladipic acid, Butanoic acid,3-hydroxy-3-methyl-, Isovaleric acid, propylester, 9,12-Octadienoicacid(Z,Z), 9,12-Octadecadienylchloride, (Z,Z), 5,6-dihydro-5-methyluracil, 9,12-Octadeca dienoicacid(Z,Z), methylester, n-Hexadecanoic acid (palmitic acid), d1-beta-phenyllactic acid (DL-alpha-Hydroxy hydrocininnamic acid), (Isovanillic acid), Hexanoic acid (Greene et al., 1996), and VaniLlic acid), ketone compounds (Gllacetophenone and Isocamphopinone), hydrocarbon compounds (1-Fluorononane) and Amides (Propanamide,2,2-dimethyl-N-(3-methylphenyl).

Table 5: Chemical Compounds of Rats' Blood Specimen Supplemented with 40mg/kg Aqueous Axttract of Leaves.

Compound Name.	Formula	M. Wt	Base ions	RT
Propanamide,2,2-dimethyl-N- (3-methylphenyl).	C ₁₂ H ₁₇ NO	191	191-57	7.684
2-Fluoro-6-(trifluoromethyl)- Acetophenone.	C ₉ H ₆ F ₄ O	206	191-163	8.263
Phenol,3,5-bis (1,1-di methyl ethyl)-.	C ₁₄ H ₂₂ O	206	191-57	8.262
5-Aminovaleric acid.	C ₅ H ₁₁ NO ₂	117	30-98	3.464
Scopolamine.	C ₁₇ H ₂₁ NO ₄	289	124-94	14.361
Hyoscyamine.	C ₁₇ H ₂₃ NO ₃	289	124-82	13.260
Atropine.	C ₁₇ H ₂₃ NO ₃	289	289-124	14.372
Apoatropin.	C ₁₇ H ₂₁ NO ₂	271	124-82	14.374
Tropacocaine.	C ₁₅ H ₁₉ NO ₂	245	140-124	14.373
Oleic acid.	C ₁₈ H ₃₄ O ₂	282	83-97	14.243
Tropic acid.	C ₉ H ₁₀ O ₃	166	91-104	7.733
3-Dimethyl-,3,4,5,6-tetra hydro-2(1H)- primidinone	C ₆ H ₁₂ N ₂ O	128	84-128	6.874
Phenol,2,6-bis (1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206	191-57	8.262
Azlaic acid.	C ₉ H ₁₆ O ₄	188	60-171	7.400
9,12-Octadecadienoicacid (Z,Z),methylester.	C ₁₉ H ₃₄ O ₂	294	81-95	13.202
2-methylpyrazine-5-Carbo xylic acid	C ₆ H ₆ N ₂ O ₂	138	138-94	15.483
Pyrrolidine,1-(2-methyl-1-propenyl)-.	C ₈ H ₁₅ N	125	110-124	14.392
Benzoyl ecgonine.	C ₁₆ H ₁₉ NO ₄	289	124-289	14.394
Isocamphopinone.	C ₁₀ H ₁₆ O	152	41-122	3.840

Compounds of Rats' Blood Specimen Treated with 40mg/kg Methanol Extract of Seeds.

The chemical compounds and their retention time, molecular formula and molecular weight are shown in table 6. The analysis using GC-MS showed 21 compounds. These compounds are as follows: alkaloidal compounds (Scopolamine, atropine, tropine, tropinone, 3-tropanone, pseudoecgonine methyl ester, apoatropin, hyoscyamine,

tropacocaine, tiglypseudotropin, benzoylecgonine, homatropine, tigloidine, aziridine, 1-methyl and tropic acid), fatty acids and esters (Oleic acid, Azelaic acid, 9,12-Octadecadienoic acid (Z,Z), 9,11-Octadecadienoic acid, methyl ester, (E,E), 9,12-Octadecadienoic acid (z,z)-, methyl ester, 6,9-Octadecadienoic acid, methyl ester and 9,12-Octadecadienoyl chloride, 9,(Z,Z) and Phenolic compounds (Phenol, 2,5-bis(1,1-dimethylethyl)- and 2,4-Di-tert-butylphenol)

Table 6: Compounds of Rats' Blood Specimen Supplemented with 40mg/kg Methanol Extract of Seeds.

Compound Name	Formula	M. wt	Base ions	RT
Scopolamine.	C ₁₇ H ₂₁ NO ₄	289	124-94	14.361
Hyoscyamine.	C ₁₇ H ₂₃ NO ₃	289	124-82	13.260
Atropine.	C ₁₇ H ₂₃ NO ₃	289	289-124	14.372
Apoatropin.	C ₁₇ H ₂₁ NO ₂	271	124-82	14.370
Tropacocaine.	C ₁₅ H ₁₉ NO ₂	245	140-124	14.373
Tropic acid.	C ₉ H ₁₀ O ₃	166	91-104	7.733
Aziridine, 1-methyl.	C ₃ H ₇ N	57	42-55	6.081
Tropinone.	C ₈ H ₁₃ NO	139	42-96	15.482
3-Tropanone.	C ₈ H ₁₃ NO	139	96-82	4.933
Pseudoecgonine, methyl ester.	C ₁₀ H ₁₇ NO ₃	199	96-199	4.930
Tigloidine .	C ₁₃ H ₁₂ NO ₂	223	124-82	14.371
Homatropine.	C ₁₆ H ₂₁ NO ₃	275	124-275	14.364
Azelaic acid.	C ₉ H ₁₆ O ₄	188	11-152	7.430
Oleic acid.	C ₁₈ H ₃₄ O ₂	282	111-83	7.433
Phenol, 2,5-bis (1,1-dimethylethyl)-.	C ₁₄ H ₂₂ O	206	191-206	7.821
2,4-Di-tert-butylphenol.	C ₁₄ H ₂₂ O	206	191-57	7.821
9,12-Octa decadienoyl chloride, 9(Z,Z)-.	C ₁₈ H ₃₁ ClO	298	81-95	13.854
6,9-Octadecadienoic acid, methyl ester.	C ₁₉ H ₃₄ O ₂	294	294-81	13.840
9,11-Octadeca dienoic acid ,methy	C ₁₉ H ₃₄ O ₂	294	95-81	13.854
9,12-Octadeca dienoic acid(z,z)-, methy	C ₁₉ H ₃₄ O ₂	294	294-81	13.822
9,12-Octadecadienoic acid(Z,Z)-.	C ₁₈ H ₃₂ O ₂	280	95-81	13.853

Compounds of Rats' Blood Specimen Treated with 40mg/kg Methanolic Extract of Leaves.

The chemical compounds of rats' blood specimen of rats treated with 40mg/kg methanol extract of leaves are shown in table 7. The GC-MS analysis showed 17 compounds. These compounds are as follows: alkaloidal compounds (scopolamine, atropine, tropine, tropinone, 3-Tropanone, pseudoecgonine methyl ester, apoatropin, hyoscyamine, tropacocaine, tiglypseudotropin, benzoylecgonine, bomatropine and bropic acid), fatty acids and esters (9,12-Octadecadienoic acid(z,z)-, ethylester, 9,12-Octadecadienoyl chloride, (z,z)- and Oleic acid, phenolic compounds (Phenol 3,5-bis(1,1-dimethyl)-, 2,4-

Ditert-butylphenol and phenol,2,6-bis(1,1-dimethyl), Ketones (2-Fluro-6-(trifluoromethyl) acetophenone), amides (Propanamide,2,2-dimethyl-N-(3-methylphenyl) and quinones (2,5-di-tert-butyl-1,4-benzoquinone, arginine and alanine).

Table 7: Compounds of Rats' Blood Specimen Treated with 40mg/kg Methanol Extract of Leaves.

Compound Name	Formula	M. Wt	Base ions	RT
2-Fluro-6-(trifluoromethyl) acetophenone.	C ₉ H ₆ F ₄ O	206	191-163	8.274
Propanamide,2,2-dimethyl-N- (3-methylphenyl).	C ₁₂ H ₁₇ NO	191	191-57	8.280
Oliec acid.	C ₁₈ H ₃₄ O ₂	282	111-83	7.431
Phenol3,5-bis(1,1-dimethyl).	C ₁₄ H ₂₂ O	206	191-206	8.272
Phenol,2,6-bis(1,1-dimethyl).	C ₁₄ H ₂₂ O	206	191-206	8.270
2,4-Ditert-butylphenol.	C ₁₄ H ₂₂ O	206	191-206	8.273
2,5-Di-tert-butyl-1,4- benzoquinone.	C ₁₄ H ₂₀ O ₂	220	205-57	12.270
9,12-Octadecadienoic acid(z,z)- , ethyl ester.	C ₂₀ H ₃₆ O ₂	308	95-81	13.861
9,12-Octadecadienonyl Chloride,(z,z).	C ₁₈ H ₃₁ ClO	298	81-95	13.862
Scopolamine.	C ₁₇ H ₂₁ NO ₄	289	124-94	14.360
Hyoscyamine.	C ₁₇ H ₂₃ NO ₃	289	124-82	13.264
Atropine.	C ₁₇ H ₂₃ NO ₃	289	289-124	14.37
Apoatropin.	C ₁₇ H ₂₁ NO ₂	271	124-82	14.374
Tropacocaine.	C ₁₅ H ₁₉ NO ₂	245	140-124	14.373
Arginine.	C ₆ H ₁₄ N ₄ O ₂	174	44-69	15.511
Tropic acid.	C ₉ H ₁₀ O ₃	166	91-104	7.731
Alanine (Amino acid). 2- Aminopropanoic acid.	C ₃ H ₇ NO ₂	89	40-44	18.150

Compounds Found only in Rats' Blood Specimen Supplemented with Methanolic Extracts of Seeds and Leaves:

Compounds detected in rats' blood that treated with methanol extract of seeds are as follows: Propanamide,2, 2-dimethyl-N-(3-methyl phenyl), 2-Fluro-6-trifluoromethyl)-Acetophenone, Phenol,3,5-bis(1,1-dimethylethyl)-,Phenol,2,6-bis (1,1-dimethylethyl), 1,3-Dimethyl,-3,4,5,6- tetrahydro-2(1H)- primidinone, Isocamphopinone and Tropic acid) while the compounds in rats' blood treated with methanolic extract of leaves are: (2-Fluro-6-(trifluoromethyl) acetophenone, Propanamide, 2,2- dimethyl-N-(3-methyl phenyl), Phenol3,5-bis(1,1-dimethyl), Phenol,2,6-bis(1,1-dimethyl)-, 2,4-Ditert-butylphenol, 2,5-Di-tert-butyl-1,4- benzoquinone and Tropic acid).

Compounds Found only in Rats' Blood Specimen Administered with Aqueous Extracts of Leaves and Seeds.

Compounds determined in rats' blood specimen treated with aqueous extract of leaves are: (2(3H)-Benzofuranone,3,3-dimethyl-5[(methylsulfonyl)oxy], P-Benzoquinone, 2,6-di-tert-

butylphenol, 2,6-bis(1,1-dimethyl)-4-methyl-, 9,11-Octa decadienoic acid, methyl ester, (E,E), 9,12-Octadecadienoic acid (Z,Z)-, 9,12-Octadecadienoyl chloride, 9,Z,Z)-, 6,9-Octadecadienoic acid, methyl ester, Arginine, 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-, Di-n-octylphthalate, (1,2-Benzenedi carboxylic acid, dioctyl ester), Acetophenone-2- carboxylic and tropic acid) while the rats' blood specimen that treated with aqueous extract of seeds contained: (Phenol, 2,5-bis (1,1-dimethylethyl)-, 2,4-Di-tert-butylphenol, Tropic acid).

4. Conclusion

From the results obtained, it was observed that the *D. innoxia* sample was very rich with benefit chemical compounds and this will attention to using the leave and seeds extracts of *D. innoxia*. The phytochemical screening of the *Datura innoxia* revealed the presence of important pharmacological bioactive substances as well as medicinal and nutritional potentials in the leaves and seeds.

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