International Letters of Natural Sciences *Online: 2014-04-29*
ISSN: 2300-9675, Vol. 14, pp 85-96
doi:10.56431/p-5cy53p

Toxicity study of *Achyranthus aspera*

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ABSTRACT

The objectives of present work focuses to check the acute and sub acute toxicity studies for the *Achyranthes aspera L* methanol extract as per OECD guideline in Swiss mice. The dose of 100, 200, 250 gm/kg body weight methanol extract by administering drug intraperitoneally and recorded the growth, body weight, organ weight, general symptoms, morphological and physiological behavior, mortality. These parameters on various organs system in mice were studied. It was considered necessary to assess its potential health hazard in man and to find the safe and effective dose. The observations of changes in body weight, food and water intake as well as cage side observations were reported. There was no abnormality observed in all groups. The whole plant powder methanol extract of *Achyranthes Aspera L* were found to be nontoxic.

Keywords**:** *Achyranthus aspera*; Toxicity; Methanol extract; Albino Rats

1. INTRODUCTION

Toxicology is the study of harmful actions of chemicals on biological tissues. It includes an understanding of chemical reactions, interactions, biological mechanisms and consequential structural changes of the tissues[1]. Study of toxicity has tremendous beneficial property form man's view point that, when the drug is at minimal concentration produces no effect and maximal concentration causes death. Therefore there must be a range of concentration of the medicine which would give a graded effect somewhere between these two extremes[2]. The experimental determination of this range of doses is the basis of the dose response relationship[3].

Neuro toxicity is another common result caused by ingestion of plant products Neurotoxicity can occur when the plant molecule acts as a blocker to neuro transmission[4]. Jimsom weed *(Datura stramonium)* has been used as tea for the treatment of asthama[5]. The atropine like substances, hyoscyamine and scopolamine occurring in all parts of the plant act to block acetylcholine which is the predominant neurotransmitter of the parasympathetic nervous system[6].

The signs and symptoms of *Datura* toxicity are manifested in many organ systems including dry mouth, dry skin and hyperexia, death can occur from cardiac arrest[7].

Achyranthes aspera L. (Amaranthaceae) is one of the plant used for medicinal purposes. It is an erect, annual herb, distributed in the hilly districts of India[8]. The plant is used in indigenous system of medicine as emenagogue, antiarthritic, antifertility, laxative,

ecbolic, abentifacient, anti-helminthic, aphrodisiac, antiviral, anti-plasmodic, antihypertensive, anticoagulant, diuretic and anti-tumor[9].

The present study of Methanolic extract of *Achyranthus aspera* was assessed to find the possible cumulative effect of acute toxicity and subacute toxicity at various levels in 30 days treatment of mice, by administering drug intraperitoneally and recorded the growth, body weight, organ weight, general symptoms, morphological and physiological behavior, mortality. These parameters on various organs system in mice were studied. It was considered necessary to assess its potential health hazard in man and to find the safe and effective dose.

Photo 1. *Achyranthus aspera.*

2. MATERIALS AND METHODS

2. 1. Collection of plant material

Whole plants of *Achyranthes aspera* were collected from Gulbarga University campus, Gulbarga during the months of October and November, 2009. After collection the plant specimens was authenticated by the Department of Botany, Gulbarga University as *Achyranthes aspera.*

2. 2. Methods of extraction

Hot extraction method using soxhlet apparatus was used for successive solvent extraction of the harvested plant materials. The whole plants collected were shade dried to complete dryness and then the material was ground to fine powder in a mixer-grinder. The 35

g of plant powder was extracted successively using solvent methanol (80 ºC) in a soxhlet. After complete extraction, the contents of each extraction were concentrated by distillation.

2. 3. Animals

Young and adult mice of Swiss albino strain weighing between 20-25 g were used and procured from the, CFTRI, Mysore. The mice were housed in a were mesh cages, five in a cage and at a temperature of 27 ±3 °C, pelleted food was supplied by Hindustan Lever Limited and tap water was given water adlibitum. The mice were made to acclimatize for one week prior to experimental use.

3. ACUTE TOXICITY TESTS[10]

To test the median lethal dose or $LD_{50,}$ the mice were given with different concentration of single dose of the drug on one occasion, on different groups of mice at that lethal dose 50 % of the mice were killed and the LD_{50} value was determined in 72 hours and for 30 days test by two routes of administration of drug i.e., oral and intraperitoneally. The mice were divided into 3 groups of 10 mice each, and all these mice were kept overnight for fasting before treatment. The first group which served as control was injected intraperitoneally with different doses of Methanolic extract of *Achyranthus aspera* at a dose rate of 150, 200, 250mg/kg body weight of the drug, respectively.

The LD_{50} at 72 hours and 30 days was calculated by using Miler and Tainter (1944) method. The mice were observed continuously for the first 2 hours and then hourly for 6 hours for any toxic symptoms and finally the number of survivors were noted upto 72 hr. and 30 days. Each group contained10 mice and approximately equal number of both sexes. The Median Lethal Dose (LD_{50}) of the extract by oral and intraperitoneal routes were calculated by following the method of Ghosh (1984). The toxicological effect was assessed on the basis of mortality and shortening of the life span of mice were observed till their natural death.

For calculation of 0 % and 100 % of death, the corrected (%) formula was used.

i.e.) % + 100 (0.25/n) and 100 % = 100 (n-0.25/n).

4. SUB-ACUTE TOXICTY STUDIES[11]

For sub-acute toxicity studies the mice were given daily dose with the lowest lethal dose of $1/12^{th}$ and $1/8^{th}$ of LD_{50} for 30 days of duration and the toxic signs were observed. The purpose of this test is to determine the maximum tolerated dose and also to study the nature of toxic reactions and to evaluate fully the toxic potential of the compound. The mice were randomly assigned into two groups of 10 mice (5 male and 5 female) each. one groups served as control and vehicle control. Remaining two groups were administered daily for 30 days intraperitoneally with approximately 25 mg/kg ($1/12^{th}$ of LD_{50} body weight and 50 mg/kg ($1/8^{th}$ of LD_{50}) body weight of the drug in adult male and female mice. Body weight of the mice was taken daily for 30 days throughout treatment. At the end of 15^{th} and 30^{th} days of treatment, mutational and differential leucocyte counts of femur bone marrow cells and hematological studies were conducted and at the end of the 30^{th} day of treatment an autopsy was performed on mice and the major organs like lungs, liver, spleen, stomach, kidney, adrenals, heart, intestine, thymus of both male and female organ weights wee noted separately. Small pieces of these different organs of mice were fixed in bouins fixative for histopathological examinations. Biochemical study was also conducted on the liver.

5. ORGAN WEIGHT [12]

The body weights were recorded on 30th day after completion of 30 days continuous treatment for different group of mice and dissected out the following organs like, lung, heart, thymus, spleen, adrenals. Stomach, intestine and kidneys. Their different wet organ weights of male and female mice were taken separately and very carefully with the help of Anadem Electronic Balance, India. Organ weight by body weight ratio in each animal was calculated by using the formula. And the average value for each treatment group was recorded.

$$\frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100 = \text{- g/100 g}$$

6. HEMATOLOGICAL STUDIES[13]

Hematological study of animals is necessary to assess the potential health hazard in man. On 15th and 30th day of the blood samples were drawn from retino bulbar venus plexus of mice with glass capillary tube to determine the total RBC and WBC count, by using hemocytometer and blood smearing method was used for differential leucocyte count and also recorded the clotting time of blood.

7. RBC COUNT[14]

The hemocytometer and its diluting pipettes were cleaned, dried, sterilized with alcohol. The glass capillary tubes and diluting pipettes were rinsed with 1 % sodium citrate solution to avoid blood clot. The blood was sucked up 0.5 mark on the RBC pipette. The stream of blood should be continuous. Thus sucked RBC is Diluted with diluting fluid with up to 101 mark o the pipette. The dilution of blood was made 200 times with diluting fluid. Precaution should be taken not allow the blood to clot. Closed the open end of the pipette by the rubber cap and mixed the blood with diluting fluid thoroughly by rotating it for 3 to 4 minutes. Blow out the fluid form the lower stem of the pipette to 0.1 mark as this is not mixed with the dilution fluid properly and is therefore not use full for experiment. Hold the pipette on the plat form of the hemocytometerslide at an angle of 45". Put a drop of diluted blood from the bulb of the hemocytometer slide at an angle of each plat from. This was to be done very carefully to avoid the blood to over flow into the groove of 'H'. A coverslip was placed on both the plat forms over the counting chambers and kept for five minutes for the blood cells to settle down. Counting of blood cells was done in 5 of the 25 central small squares if the hemocytomter. For convenience sake 4 small squares at the four corners and a central small square were selected for counting of RBC. Each of these 5 squares are further divided into 16 smaller squares. Hence the counting was done in 80 small squares (16 x per square was calculated by using the formula.

$$\frac{\text{Total no. of cell counted x dilution x } 4000}{80} = \text{million cells/cu mm of blood}$$

7. 1. WBC count[15]

It is similar to the method used for the total count of RBC but with slight difference. Here WBC pipette was used in place of RBC pipette and the blood was sucked up to 0.5, mark on the pipette. Sucked WBC is diluted with diluting fluid up to 11 mark on the pipette. The dilution of this blood was 1 part of WBC and 20 part diluting fluid, while counting the WBC. The light was kept at minimum and counting of WBC was done in two of the four counting chambers. Each of these four WBC counting chambers is of 1 sq mm area and the number of WBC cells per cubic milli meter was calculated by using the formula.

$$\frac{\text{No. of WBC's counted x dilution x10}}{\text{4 x No. of chambers counted.}} = \text{cells/cu mm}$$

7. 2. Differential Leukocyte Count[16]

A drop of blood was smeared on to a clean and dry slide to get a thin and uniform film of blood on the dry slide. The thin film of blood was dried by waving the slide in air. Later on the slide was dipped in Fleishman buffer to avoid the shrinking of leucocytes cells and then stained with Fleishman dye. The blood film was examined under higher magnification (40 X) with oil immersion to count the differential leucocytes such as lymphocytes, coenocytes, neutrophils, basophiles and eosinophils. The percentage of leucocytes was calculated as 500 leucocytes cells per animal.

7. 3. Determination of clotting time of Blood[17]

A drop of blood was dropped on a clean slide and immediately the time was noted. A clean and sterilized needle was moved slowly through the drop of blood, when a fine thread of fibrin can be pulled up by the needle, it is known that coagulation has started and the time was noted.

7. 4. Statistical analysis

The data on the different parameters of sub acute toxicity studies were evaluated by student 't' test. The significant value was considered at $p \leq 0.05$. By using the following formula:

$$T = \frac{x1-x2}{\sqrt{SE2\ 1+ SE22}}$$

The obtained't' value and degree of freedom (n-1) were correlated with't' distribution table and found out the p value.

8. RESULTS AND DISCUSSION

8. 1. Acute toxicity studies

1000 mg/kg and 500 mg/kg body with of the drug was administered intraperitoneally for 2 groups of 10 mice each. Observed continuously for 6 hours did not show any toxic symptoms. Within 24 hours all these mice have shown the toxic symptoms like weight loss, reduced food and water intake, poor response to external stimuli, drowsiness, lethargy, fifing of hair, paddling of the feet, sluggishness, about 20 % of the mice died when treated with 1000 mg/kg body weight but no death was observed in 500 mg/kg body weight and but all these mice (100 %) died within 36 hours of treatment. 250, 200 and 150 mg/kg body weight of the drug treatment net also showed similar types of toxic symptoms. At all these dose

levels, 100 % of the death of mice was observed within 60 hours. (Table 1). There was a gradual increase in the survival percentage of mice with gradual decrease in the drug dose over a period of 72 hours of treatment. The same experiment was continued for a period of 30 days. The mice did not show any changes in the percentage survival, except at 250 mg/kg body weight of the drug treated group of mice showed 30% death (Table 2) as compared with the results obtained at 72 hours.

For acute dose of 200 mg/kg body weight of Methanolic extract of *Achyranthus aspera* given intraperitoneally was tolerated by mice without any apparent adverse manifestations except for lethargy and ruffling of air. The percent mortality in each group was converted in to probit value and plotted against log dose for 72 hours and 30 days the graph revealed that LD_{50} was 200 mg/kg body weight (Table 1 and 2). When the mice were given orally at 250 and 500 mg/kg body weight of the drug, they did not show any toxic symptoms or mortality as compared to intraperitoneal administration. This may be because the drug was absorbed by the gastrointestinal tract or it might have detoxified by the liver.

Table 1. Acute intraperitoneal toxicity (72 hours mortality) of Methanolic extract of *Achyranthus aspera* (MEAa) in Swiss albino mice.

Group	Dose (mg/kg)	Log dose	Dead/Total	Dead (%)	Corrected (%)	Probit value
1	150	1.68	0/10	0	2.0	2.62
2	200	1.80	0/10	0	2.0	2.60
3	250	1.90	2/10	20	20	3.65

Table 2. Acute intraperitoneal toxicity (30 days mortality) of Methanolic extract of *Achyranthus aspera* (MEAa) in Swiss albino mice.

Group	Dose (mg/kg)	Log dose	Dead/Total	Dead (%)	Corrected (%)	Probit value
1	150	1.68	0/10	0	1.9	2.60
2	200	1.80	0/10	0	1.9	2.62
3	250	1.90	3/10	30	30	3.98

9. SUB-ACUTE TOXICITY STUDIES

9. 1. General symptoms and body weight

Sub-acute toxicity studies of Methanolic extract of *Achyranthus aspera* revealed that the mice did not show any external manifestation of toxic symptoms, other behavioral patterns and mortality even after 30 days of drug administration (25 mg/kg/day and 50 mg/kg/day). It did not produce any statistically significant changes in the body weights (Table 3), food and water intake, hematological, bone marrow studies of differential leucocytes count and chromosomal morphology. But the present study also revealed that the significant changes were found in biochemical, histopathological and weight of the few major organs etc.

Table 3. Weekly average body weight of mice during the curse of 30 days of administration of Methanolic extract of *Achyranthus aspera.* (MEAa).

Treatment	Dose mg/kg	Weekly body weight (in g)				
		Initial	I	II	III	IV
Control	---	22.66±0.372	22.92±0.335	24.5±0.303	25.92±1.095	25.92±0.944
V.con	Dw	22.20±0.326	22.60±0.418	24.10±0.64	25.00±0.523	25.80±0.489
MEAa	25	23.00±0.229	23.92±0.0300	25.25±0.484	26.38±0.920	26.75±0.318
MEAa	50	23.42v0.367	22.92±0.618	24.58±0.424	25.98±0.712	25.83±0.566

9. 2. Organ weight

On 30th day the macroscopic examination of the major organs such as lungs, liver, kidney appearance of colour and size. But considerable enlargement (almost double the size) of spleen was observed when compared with control group of mice. It is evident from the Table 4 that the drug at 25 mg/kg and 50 mg/kg body weight has shown either increase or decrease in the weight of organs and the drug at these two doses acted differently in both the sexes.

It is found to have no significant effect on the adrenals and heart in both the sexes at both the doses of drug indicating drug indicating the safety of the drug on these organs. However these drug doses have noticeable effect by decreasing in the weight of the lungs in both the sexes and at both doses. However the significant decrease in the weight of the lungs was noticed only in female mice at 50 mg/kg body weight. On the contrary there was an increase in the weight of the stomach in both the sexes at both the doses. More in weight was noticed again at higher dose in both the sexes.

The lower dose has brought about significant increase in weight of the intestine in male mice but no significant increase in female mice at lower dose. But at higher dose there was decrease in the weight of the intestine in both the sexes. Similarly an increase in the weight of the pancreas was noticed in both the sexes at lower doses but at higher dose there was decrease in weight of the pancreas was noticed.

The gradual decrease in the weight of the liver in male mice as the drug dose increased in contrast with gradual increase in the weight of the liver of the female mice when the drug dose was increased. Thus drug behaved differently with the spleen and thymus. There was an increase in the weight of both the organs in male at both the doses. But there was significant increase in weight of the spleen in female only at higher dose of 50 mg/kg rather than at 25 mg/kg body weight (Table 4). But these changes of organs weight did not have any adverse effects on the general growth and behavioural activity of the mice. Except the enlargement of spleen induces toxic inflammatory reactions in mice.

Table 4. Organ weight in albiono mice after 30 days of treatment with Methanolic extract of *Achyranthus aspera* (MEAa) (Organ weight expressed in g/100 gm body weight).

Organs	Sexes	Distilled water treated	25 mg/kg/drug	50 mg/kg drug
Liver	Male	6.218±0.545	6.06±1.414	5.969±0.685
	Female	5.975±0.246	6.03±0.706	6.264±0.187
Spleen	Male	0.548±0.06	0.568±0.141	0.572±0.350
	Female	0.645±0.002	0.633±0.448	0.858±0.028a
Lung	Male	0.736±0.002	0.716±0.392	0.114±0.098
	Female	0.938±0.071	0855±0.035	a0.719±0.0134
Heart	Male	0.617±0.053	0.626±0.042	0.618±0.0437
	Female	0552±0.0207	0.549±0.008	0.564±0.0157
Pancreas	Male	0.503±0.387	0.522±0.071	0.466±0.0467
	Female	0.526±0.009	0.535±0.014	0.41±0.084
Intestine	Male	13.254±0.179	14.604±0.43a	13.099±1.654
	Female	13.144±0.102	13.378±0.44	12.821±0.584
Thymus	Male	0.040±0.009	0.042±0.003	0.043±0.007
	Female	0.038±0.0085	0.038±0.008	0.042±0.001

Note : a = Significant when compared to control (a = $P < 0.05$)

On 30th day the detailed post mortem examination has shown that the major organs like lungs, liver, stomach, heart did not show any pathological changes even after 30 days of treatment with Amalakyadi churna at 25 and 50 mg/kg body weight. Only at 50 mg/kg there were few darkly stained nuclei in the villi of intestine, medullary region of the kidney and spleen was observed (Plate-VII a, b and c) indicating mild damage occurred in these tissues, but these organs did not show any other deformities. This study also revealed that the drug did not induce any hepatotoxicity.

10. HEMATOLOGICAL STUDIES

10. 1. Total RBC and WBC count

The administration of Amalakyadi churna extract has shown slight increase in the total number of RBC cells but statistically not significant and no change in the total number of WBC cells on the 15th and 30th days (Table 5).

Table 5. The peripheral blood changes in mice treated for 15 and 30 days with Methanolic extract of *Achyranthus aspera*. (MEAa).

Treatment	Dose (mg/kg)	RBC million/cu mm		WBC cells/cu mm	
		15th day	30th day	15th day	30th day
Control	--	2.90±0.0534	7.65±0.5	46.66±0.7	47.4±0.61
V.com	Dw	2.70±0.707	46.74±1.2	47.9±1.2	47.9±0.93
MEAa	25	3.71±0.0799	7.62±0.2	46.72±.2.0	47.9±0.93
MEAa	50	3.34±1.2089	7.75±0.9	49.66±0.8	47.3±0.42

10. 2. Total differential leucocyte count

The administration of Methanolic extract of *Achyranthus aspera* has shown sight change in the differential leucocyte cells count. There was slight increase in the number of neutrophils and simultaneously decrease in the percentage of lymphocyte cells. No changes in the eosinophils and monocyte cells at 25 mg/kg on 15th and 30th day of observation in both the sexes, these changes are not statistically significant.

Whereas at 50 mg/kg treated group of mice, there was increase in the number of monocyte, eosinophils, neutrophils and decrease in the number of lymphocyte cells were observed on 15th and 30th day in both the sexes. The statistical analysis revealed that these changes are not significant. There was no sex biased difference found (Table 6). 50 mg/kg drug dose induced the enlargement of the spleen size and neutrophil count. This enlargement of spleen and increase in neutrophoil count indicate the toxic inflammatory reactions (Robins, 1974).

Table 5. The differential leucocytes count of blood in mice after treatment for 15 and 30 days with Methanolic extract of *Achyranthus aspera*. (Number of cells expressed percentage).

Treatment	Dose (mg/kg)	15th day					30th day				
		Mo	Ne	Eo	By	Ba	Mo	Ne	Eo	By	Ba
Control	--	2.0± 0.199	14.4± 0.317	0.5± 0.109	8.3± 0.849	0	2.6± 0.46	15± 0.02	0.6± 0.002	81.8± 0.853	0
V.com	Dw	2.5± 0.323	16± 0.319	0.5± 0.024	80± 0.024	0	2.8± 0.087	14.9± 0.762	0.6± 0.106	80.6± 0.576	0
MEAa	25	3.0± 0.141	16.4± 0228	0.4± 0.001	80± 7.086	0	3.6± 0.303	15.2± 0.750	0.6± 0.106	80.6± 0.576	0
MEAa	50	5.2± 0.909	18.8± 1.053	0.8± 0.006	75.2± 2.378	0	8.0 1..01	17.8± 0.944	1.0± 0.001	73.2± 1.533	0

10. 3. Clotting time of blood

The 15th and 30th day of treatment, the drug did not show any changes in clotting time of blood in mice as compared with the control group of mice (Table 7).

Table 7. Clotting time of blood after 15 and 30 days of treatment with Methanolic extract of *Achyranthus aspera*. (MEAa).

Treatment	Dose (mg/kg)	15th day (clotting time in seconds)	30th day (clotting time in seconds)
CONTROL	--	119.5±0.354	120.5±0.204
V.com	dw	119±0.0707	119.5±3.354
MEAa	25	120±0.726	116.8±0.509
MEAa	50	119.6±0.334	120.5±0.612

11. BONE MARROW STUDIES

11. 1. Differential leucocytes count

The treatment at 50 mg/kg/day and 25 mg/kg/day of Methanolic extract of *Achyranthus aspera* given continuously for 30 days and studied on 15th and 30th day. This drug did not bring any significant changes in total number of the differential leucocytes in the femur bone marrow cells of mice.

11. 2. Chromosomal aberration

The Table 8 shows that there was no significant change in the chromosomal morphology in both the treatment groups on 15th and 30th days of observation. It is evident that the drug did not induce any chromosomal mutations or changes in chromosomal morphology like fragmentation, chromosomal and chromatid breads, ring formation, dicentrics and polypoloidy. The earlier work on plumbag in by Santhakumari and Radhakrishna Pillai (1980) have studied the effect of plumbagin on cell growth and mitosis and shown that plumbagin is a powerful cytotoxic, hepatotoxic and nucleotoxic compound which behaves like a spindle poison by inhibiting entry of cells into mitosis even with a concentration as low as 0.1 μg.

Table 8. Chromosomal abnormalities after 15 and 30 days of treatment with Methanolic extract of *Achyranthus aspera* (MEAa) (Expressed I %).

Treatment	Chromosomal aberrations						
	Fragments	Dicentrics	Rings	Chromosome Breaks	Chromatid breaks	Polyploidy	SDS
Control (no)	0.42± 0.0141	-	-	0.023± 0.097	0.15± 0.1149	0.00± 0.00026	-
V.cm (dw)	0.405± 0.0035	-	-	0.03± 0.007	0.25± 0.0354	0.0025± 0.00018	-
MEAa (25 mg/kg) 15th day	0.395± 0.0106	-	-	0.045± 0.0035	0.2± 0.0707	0.0028± 0.00043	-

MEAa (25 mg/kg) 30th day	0.405± 0.0286	-	-	0.031± 0.0014	0.33± 0.01767	0.0038± 0.0004	-
MEAa (50 mg/kg) 15th day	0.39± 0.02765	-	-	0.04± 0.00707	0.3± 0.0707	0.006± 0.0071	-
MEAa (50 mg/kg) 30th day	0.41± 0.0298	-	-	0.035± 0010	0.3± 0.0414	0.005± 0.0007	-

12. CONCLUSION

The herbs occupied a distinct place in the life right from the primitive period till date and provided information on the use of plants or plant products and products as medicine. The use of medicinal plants in the management of various illnesses is due to their phytochemical constituents and dates back antiquity. It is seen from the literature that *Achyranthes aspera* is a very important plant for its large number of medicinal properties as well as medicinally important chemicals like ecdysterone, achyranthine, betaine, pentatriaontane, 6-pentatriacontanone, hexatriacontane and tritriacontane.

The plant shows many pharmacological activities like spermicidal, anti-allergic, cardiovascular, nephroprotective, antiparasitic, hypoglyceamic, analgesic and antipyretic. Many traditional uses are also reported like antiperiodic, purgative and laxative, in various types of gastric disorders and in body pain which are being studied till today and further research has to be done. Thus, *Achyranthes aspera* is quite promising as a multipurpose medicinal agent so further clinical trials should be performed to prove its efficacy.

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(Received 22 April 2014; accepted 27 April 2014)