Chromosomal Aberration of Fasciola Hepatica Acetone Powder: In Vitro Study on Human Lymphocytes

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Abstract

Fasciola hepatica metazoan trematodes parasite that is living in the liver of their host (sheep, goat and human). It usually cause pathological changes in the parasitized organs. Acetone powder technique is used to prepare partially purified organic material of adult worms. Various concentrations of Fasciola hepatica acetone extract (5 mg/ml, 10 mg /ml , 15mg/ml , 20 mg/ml and 40 mg /ml) are used on human lymphocyte culture for studying some chromosomal aberration. Chromosomal aberrations includes Isogap, Breaks and Dicentrics for chromosomes; Gaps and deletion for chromatids; and mitotic index (MI). This In-vitro study revealed that acetone powder extract of Fasciola hepatica parasites causing various genotoxicity effects in the human lymphocyte cells. These effects are ascending in their increasing in significance differences ($P< 0.001$) for all treatment against control as concentration of acetone extract is increased.

Keywords: *Fasciola hepatica, acetone powder, chromosomal aberration*

Introduction

Fascioliasis according to WHO (The World Health Organization) account in the list of the Neglected Tropical Diseases (NTDs), in the collection of food-borne trematodiases [1]. This illness is a parasitic zoonosis caused by pair of hepatic fluke which are Fasciola hepatica distributed throughout Europe, Africa, Asia, Oceania and the Americas, and Fasciola gigantica limited to some area of Africa and Asia [2]. Infecting is widely distributed in livestock, human. Infection by Fasciola species was considered of minor importance till 1990 [3]. Human influences by these Trematodes start to display its significance from the next decade, with the increasing of its report of several human endemic regions and an elevation of human infection cases reports [4]. Fascioliasis is chiefly a rural disease because human infection hazard is in the arena where the disease transmission occurs in freshwater accumulation inhabited by the lymnaeid helix vectors [5]. The impression of this disease is due to its great pathogenicity [6]. Also, immune suppression which occur not only throughout the migratory, invasive or acute phase [7], as mentioned before [3]. This is observed as well as through the long biliary and or chronic phase [8], in which nearly all of the inhabitants of human endemic capacities are diagnosed [4], and also where in the reinfections in human hyperendemic places [9].

Many parasites exhibited effects on the chromosomes of their host's cell. These effects occur as chromosomal aberrations either In Vitro or In Vivo [10].

Current study is design to investigate these chromosomal aberration In-vitro in the presence of Fasciola hepatica extract for the first time.

Material and Methods

1- Fasciola hepatica parasitic worms were collected from local abattoir, and identified according to their

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morphological feature as well as the dependent scientific key mentioned previously [11].

2- The worms account to be in about 50 grams in weigh. Washed three times with standard sterile PBS. Minced with number 3 sterile scalable in sterile ceramic tray. After that they homogenized with sterile glass homogenizer. The homogenate filtered with sterile number 42 filter paper upon sterile Buchner funnel.

3- Acetone powder prepared from the precipitation of above step by using 1:10 of weight to volume against cold standard acetone (- 18 ºC) for three times. Lately the powder but in (25 ºC) overnight for complete drying. This procedure is adapted from various authors [12, 13]. This protocol applied for the first time on parasitic Fasciola hepatica worm with current study.

4- Various concentration of above prepared Fasciola hepatica acetone powder are used (sterile PBS) against Human lymphocyte chromosomal preparation. The maximum solubility and concentration give 50% toxicity of culture cell was shown to be 400mg/ml, and doses account according to that by dividing this worm's acetone extract by 10 and treatment doses will be (5, 10, 20, and 40 mg / ml) of used medium. This protocol was used previously [14], and modified here according to get series doses.

5- Human lymphocyte was prepared and results analyzed according to Proudlock and Bisen [13, 15]. Three healthy young men aged 22-25 years have donate their blood. Studied cells are 1500 cells for 3 times repeated reading in triplicate of each used concentration.

6- Statistical analysis was done according to recommendation of Kirkland and Mills [16, 17].

Results and Discussion

Fasciola hepatica considered one of helminthes trematodes that had zoonotic approach in their infection [17]. This parasite occupied liver parenchyma in the primary infection and then resident in biliary ducts of liver [6]. The parasite morphology exhibited uncompleted digestive system which certainly exchange its wastes with host organ cell [18]. Fascioliasis shows many clinical signs s especially in chronic type, like hypertrophy of liver ducts, biliary ducts, cirrhosis, and some time carcinogenic effects

[19, 20, 21]. Chromosomal aberration in the living systems indicate reaction of cell chromosomes against forging substances [22, 23], and that include parasitic biological one [24]. As this study aim to investigate this probability for the first time in a new approach by using acetone powder prepared from adult worms against human lymphocytes chromosomes in In vitro. Fasciola hepatica acetone powder revealed various genotoxicity as showed by table one. All reads showed to be significant ($P<0.001$) for all treatment in compare with control.

These Chromosomal aberrations and Mitotic Indices in human lymphocyte cells treated with different concentrations of Fasciola hepatica acetone powder (Tab.1) are seen to be ascending with increasing of prepared acetone powder. In investigation of chromosomal aberrations which includes isogap, breaks and dicentrics for chromosomes and gap and / or deletion for chromatids.

It is clear that there is no clear effect of sterile PBS (Phosphate Buffer Saline) in control treatment. In (5 mg / ml) of acetone powder these chromosomal aberration be (All reading in percent of total studied cell) 13.1, 5.9 and 0.5 respectively, then in 10 mg /ml they are increased to be 53.2, 17.1 and 21.8. Also in 20 mg / ml they are increased to be 49.5, 40.7 and 55.2. In the last used concentration the aberration reads 66.7, 51.3 and 61.1.

These results reflects that Fasciola hepatica acetone powder have genotoxicity effect leads to these chromosomal aberration like many organic substances derived from other organism like plants with other methods of extraction [25] or for Amoeba parasite [26] but not by acetone powder method. These effects may explain in some manner how carcinogenic property of chronic infection with Fascioliasis above liver of their host which may be related to release of toxic parasite substances through various growing phases of it in

parasitized location as for liver in case of liver fluke here. In the study of chromatids aberrations which are related to chromosomal aberration also but may be considered as internal genotoxic to chromosomes structure [27, 28]. It appear that gap and deletion occurs in the same feature of chromosomal aberration as they are increased in ascending style according to increasing of acetone powder concentration to be 10.1 and 5.4 respectively in control slides, then 45.2 and 15.23 for next concentration. Flowed by 177.15 and 33.1, 201.16 and 57.9, lately to be 199.3 and 80.2 (All reads at percent of total investigated cell also). Mitotic Index which representing number of cell at dividing stage among total cells that have been studied in relation with various used treatment (Tab.1).showed an increasing from control to higher used concentration also, and that be in percent (%) 6.1, 7.3, 7.5, 8.1 and 8.9 in the last one [29].

Table 1 – Chromosomal aberrations, and Mitotic Indices in human lymphocyte cells treated with different concentrations of Fasciola hepatica acetone powder (mg/mL).

Treatment (Fasciola hepatica acetone powder – mg/ml)	Chromosomal aberrations			Chromatids aberrations		Mitotic Index (%)	Number of Analyzed cells	Number of samples (after 48 hrs. of culturing)
	Isogap (%)	Break(s) (%)	Dicentrics (%)	Gap (%)	Deletion (%)			
Control	0	0	0	10.1	4.5	6.1	1500	3
5	13.1	5.9	0.5	45.2	15.23	7.3	1500	3
10	53.2	17.1	21.8	177.15	33.1	7.5	1500	3
20	49.5	40.7	55.2	201.16	57.9	8.1	1500	3
40	66.7	51.3	61.1	199.3	80.2	8.9	1500	3

Comparison of total chromosomal aberrations vs. chromatids aberrations according to various Fasciola hepatica acetone powder concentration (fig.1) shows that chromosomal aberrations are less than chromatids aberration in compare with control slides and increasing with various used Fasciola hepatica acetone powder concentration ($P<0.001$). These are 0, 19.5 , 92.1 , 145.4 , 178.1 for chromosomes vs. 15.5 , 60.4 , 211.1 , 259.3 and 279.4 for chromatids as seen in tab 1 orderly.

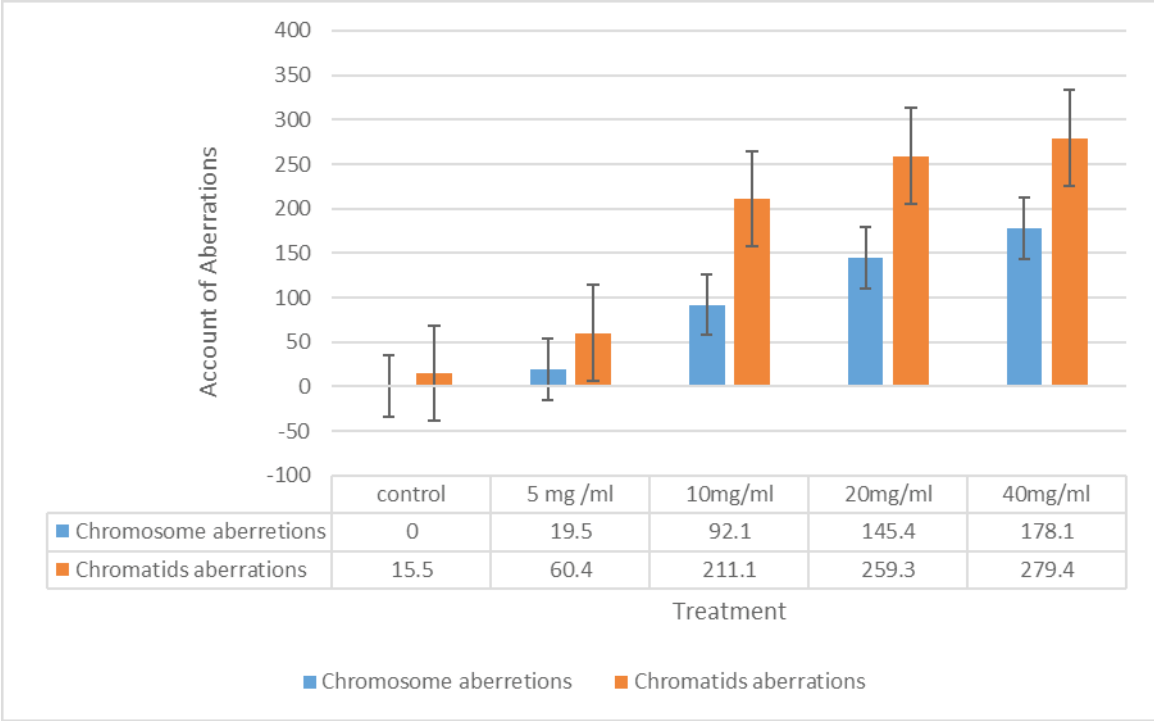

Fig. (1): Comparison of Total chromosomal aberrations vs. chromatids aberrations according to various Fasciola hepatica acetone powder concentration (All treatments are significant at P<0.001 vs. control).

Comparison chromosomal aberration types according to various Fasciola hepatica acetone powder concentration. Fig.2 shows significant (P<0.001) distribution in their occurrence for Isogap, break and dicentrics but not in control groups. This result clarified that the first type of these chromosomal aberration type is Isogap then break and lately dicentrics as well as the concentration increased.

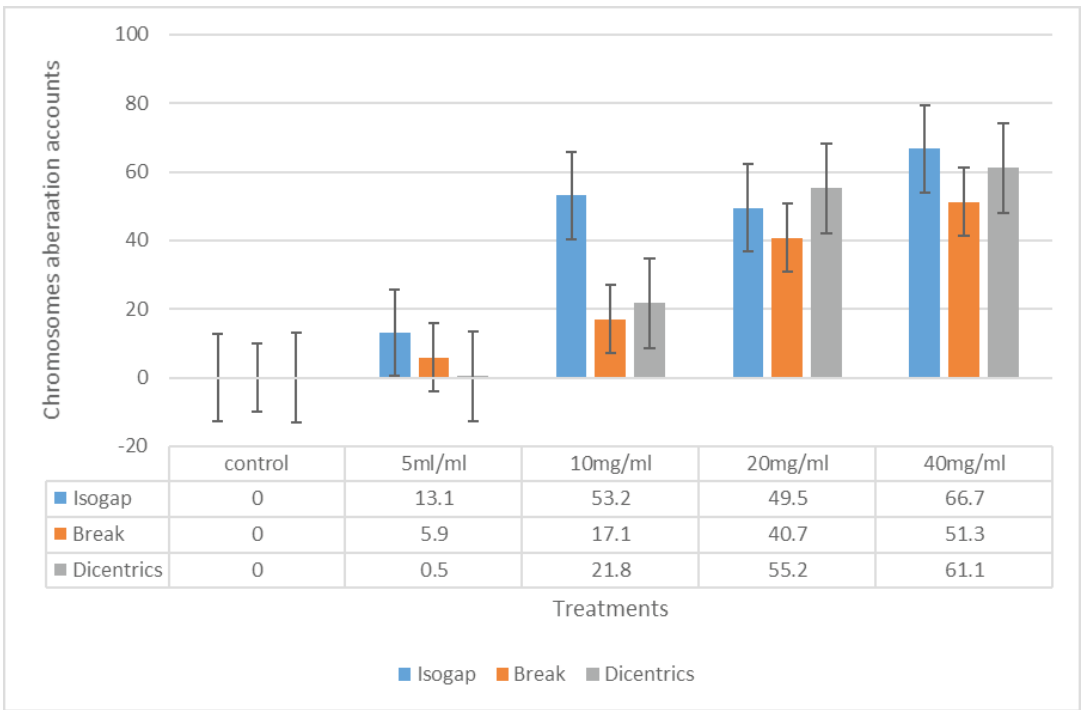

Fig. (2): Comparison chromosomal aberration types according to various Fasciola hepatica acetone powder concentration (All treatments are significant at P<0.001 vs. control).

In figure 3 , Comparison chromatids aberrations types according to various Fasciola hepatica acetone powder concentration results are arranged in the same style as for chromosomes aberration for their occurrence in significance (P<0.001) to increasing of acetone powder concentration .

These chromatids aberration are gap and deletion. Gaps of chromatids are seen to be more in their observation in compare to deletion in respect of applied acetone powder concentration. These results are compatible with previous studies in other organisms [30, 31].

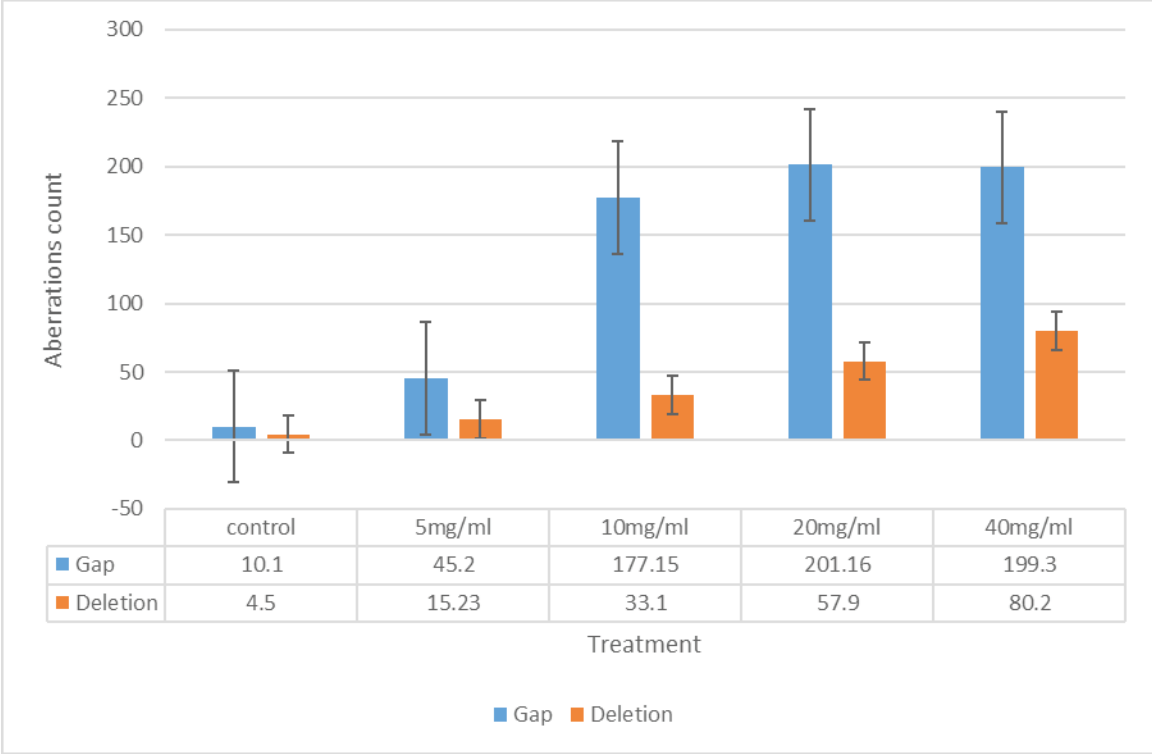

Fig. (3): Comparison chromatids aberrations types according to various Fasciola hepatica acetone powder concentration (All treatments are significant at P<0.001 vs. control).

Conclusions

Fasciola hepatica acetone powder which prepared in this research for the first time shows various chromosomal aberration and that may be help in explanation of some phases of this parasite pathogenicity and genotoxicity.

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Ethical Clearance: Not required

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