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Author(s)	KIMURA, Yanagi
Citation	北海道大學理學部紀要, 15(2), 264-271
Issue Date	1963-03
Doc URL	https://hdl.handle.net/2115/27371
Type	departmental bulletin paper
File Information	15(2)_P264-271.pdf

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The Cytological Effects of Chemicals on Tumors, XXI. Notes on the Effects of Crude Extracts from Japanese Podophyllaceous Plants on Transplantable Rat and Mouse Ascites Tumors[1)]

By

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(With 8 Text-figures and 1 Plate)

The search for potential tumor-damaging agents in higher plants has been stimulated by the findings that colchicine, podophyllin and their constituents were capable of producing remarkable regression and cytolysis of tumors. Programs for screening plant extracts for possible anti-cancer activity have been carried out in various laboratories with tumors transplanted in mice and rats, with egg-cultivated tumors and with tumors developed spontaneously in experimental

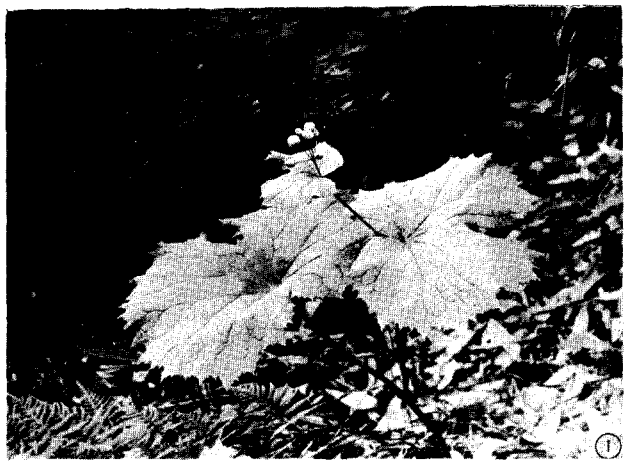


Fig. 1. *Diphylleia grayi* with fruits (×1/7).

1) Contribution No. 609 from the Zoological Institute, Faculty of Science, Hokkaido University. Supported by a grant from the Damon Runyon Memorial Fund to S. Makino for Cancer Research (DRG-563C (T)).

animals (Belkin and Fitzgerald 1952, 1953a, b, Belkin *et al.* 1952, Taylor *et al.* 1952, 1956, Fitzgerald *et al.* 1953, 1958, McKenna *et al.* 1954, 1958, 1959, 1960, 1961, Weisberger and Pensky 1957, 1958, Dalal *et al.* 1958, French *et al.* 1961, Hardinge *et al.* 1961, 1963, Leiter *et al.* 1961, 1962, Matthies 1961, McKenna and Taylor 1962, Grunberg 1963). Although the number of plants processed to date is but a small part (about 4,000 species) of the Plant Kingdom, further extensive studies of this sort seem to be of importance for therapeutic influence on cancer.

The present investigation was undertaken with the purpose of observing possible antitumor activity of Japanese Podophyllaceous plants, *Diphylleia grayi* (Fig. 1) and *Glaucidium palmatum*, from a cytological standpoint. A well-known 'Podophyllin' was isolated from American and Indian Podophyllaceous species (Biesele 1958). The systematic positions of the above species are as follows:

Family Podophyllaceae
- Subfam. Podophylloideae
  - *Podophyllum peltatum* (mandrake, or may apple)
  - *Podophyllum emodi* (Indian mandrake)
  - *Diphylleia grayi*
- Subfam. Glaucidioideae (monotypic)
  - *Glaucidium palmatum*

The author is grateful to Professor Sajiro Makino for valuable suggestion and advice, and to Drs. S. H. Hori and T. Matsuzawa for thier kind aid in preparing the extracts and for valuable advice. Special thanks are expressed to Dr. H. Toyokuni, for identifying and supplying the plant materials with appropriate suggestions.

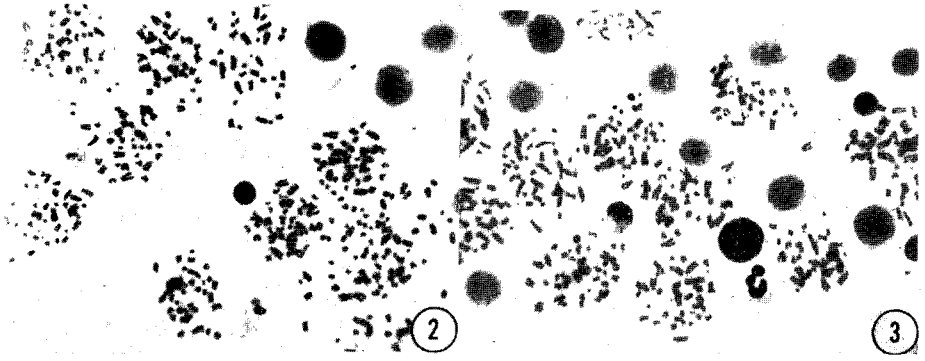
Materials and Methods

Plants used in the present experiments were *Diphylleia grayi* and *Glaucidium palmatum* collected on Zenibako Pass (Prov. Shiribeshi), on Mt. Yupari (Prov. Ishikari), and on the Taisetsu Mts. (Prov. Ishikari) during June to September, 1963. They were dissected into small pieces, ground with quartz sand, shaken with the same volume of saline solution, and then left at 10° for 24 to 30 hours. After centrifugation for 10 minutes at 3,000 r.p.m., the supernatant was collected by decantation and evaporated to dryness under reduced pressure in a freezing-drying machine. Tumors used for this study were MTK-sarcoma III and Yoshida sarcoma in Wistar rats weighing 110 to 160 grams, and Ehrlich ascites carcinoma in AKR, dd and Swiss mice weighing 20 to 25 grams. The saline extracts were dissolved in physiological saline solution at doses of 1, 3, 5 and 10 mg per 100g body weight, and injected into the peritoneal cavity of animals starting on the third or fourth day of tumor transfer. Observations were made at 3-hour intervals in the first 24 hours after treatment, at 6-hour intervals in the next 24 hours and 24-hour intervals in the following 48 hours, according to the acetic dahlia squash technique (Makino 1957). The untreated controls and animals treated without benefit survived 8 to 9 days in rats bearing the MTK-sarcoma III, 12 to 14 days in those bearing the Yoshida sarcoma, and 14 to 16 days in mice bearing the Ehrlich ascites carcinoma.

Results of Observations

The MTK-sarcoma III, Yoshida sarcoma and Ehrlich carcinoma responded similarly to the extracts. Hence, the following descriptions apply to all cases. The present article was based on the results obtained from 88 experimental animals in comparison with the results from 16 rats treated with 10 to 20γ per 100 g of colchicine, and those from 24 tumor-bearing animals used as controls.

Effects of the extract from *Diphylleia grayi*: In early treatments, the extract acted as a mitotic poison, such as colchicine, resulting in blockade of mitosis at metaphase (Figs. 2, 3 and 10), and shortening and scattering of chromosomes. In each



Figs. 2 and 3. Blockade of mitosis at metaphase. Six hours after injection of the *D. grayi* extract. 2, MTK-sarcoma III. 3, Yoshida sarcona. (acetic dahlia). [× 400].

case, the most marked effect was found 6–9 hours after injection, during which period mitotic incidence of tumor cells became 11–50% (Figs. 4–6). Meanwhile, the mitotic index of untreated cells was 3–3.5% and that of colchicine-treated cells

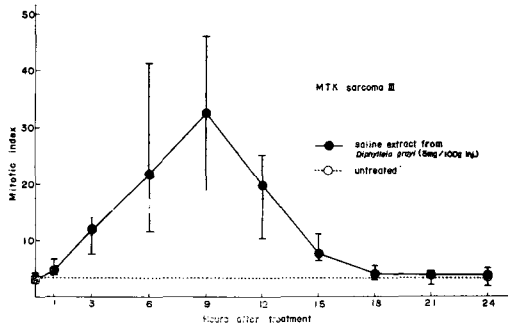


Fig. 4. Curve showing change of mitotic index of MTK-sarcoma III following injection of the *D. grayi* extract.

was 8–15%. In addition, percentages of metaphase cells in total mitotic cells were 85–95% in treated cells, while those in untreated cells were 50–60% (Fig. 7). These figures clearly indicate anti-mitotic effects of the extract. The extract from *Diphylleia grayi* is far superior to colchicine in mitotic damage. At a later time following injection of the extract, the chromosomes became shorter and

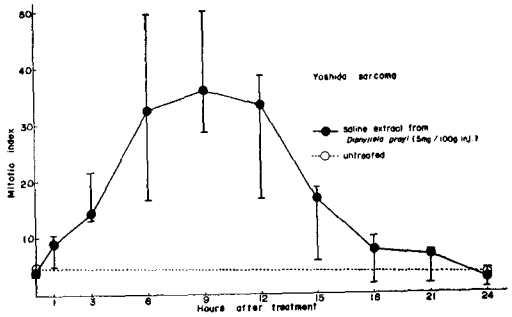


Fig. 5. Curve showing change of mitotic index of Yoshida sarcoma following injection of the *D. grayi* extract.

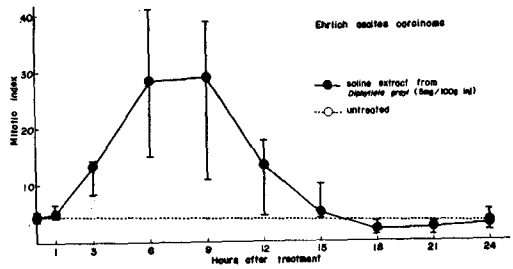


Fig. 6. Curve showing change of mitotic index of Ehrlich carcinoma following injection of the *D. grayi* extract.

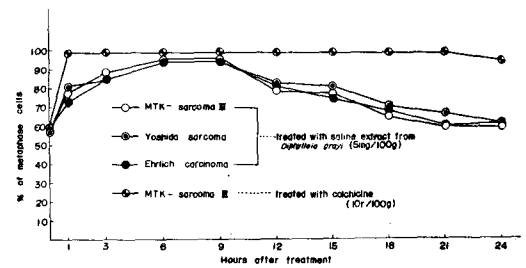


Fig. 7. Curves showing percent of metaphase cells of ascites tumors following injections of the *D. grayi* extract and of colchicine.

more irregularly thickened. At a still later stage they appeared as unusually deformed, rounded or bizarre bodies, producing pycnotic aggregation of chromatin; some of the condensed chromosomes were agglutinated into irregular masses of different size and shape, and scattered in the cytoplasm. The majority of affected cells died without proceeding beyond metaphase. More than 70% of the tumor cells were undergoing degeneration. It was found that the higher the doses, the greater the damage produced, but the response pattern of tumor cells was similar in different doses of the extract. After such severe degeneration of tumor cells a large number of inflammatory cells appeared in the ascites, and subsequently the tumor cell debris disappeared from the ascites (Fig. 14). It is evident from the above findings that the tumors regressed drastically by the action of the extract. The process of cell disintegration after treatment with the extract closely resembles that of podophyllin-treated cells (Makino and Tanaka 1953).

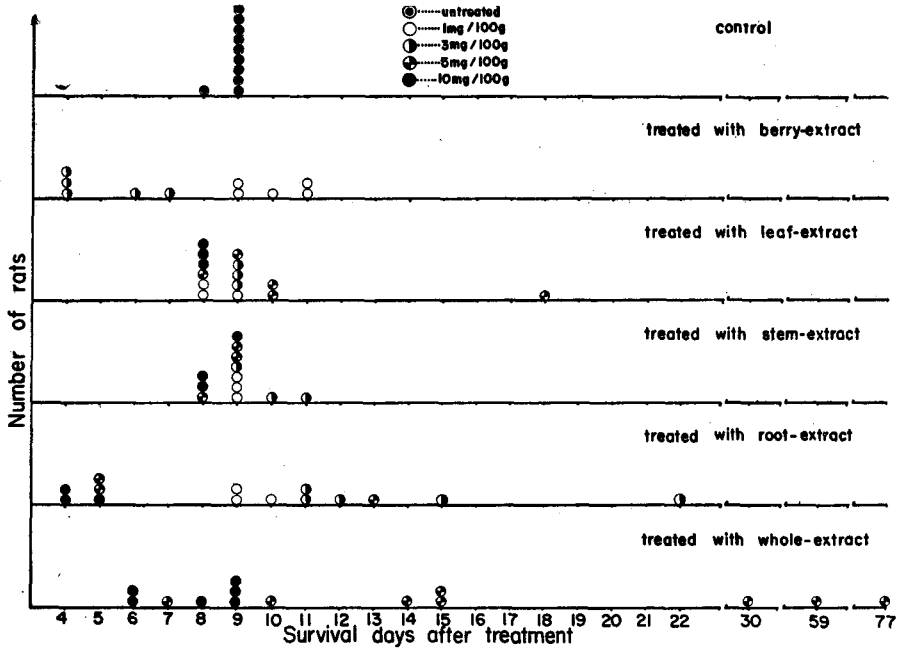


Fig. 8. Graph showing life span of rats bearing the MTK-sarcoma III following injection of the *D. grayi* extract.

The life span of treated rats bearing the MTK-sarcoma III is shown in Figure 8. It is apparent that the prolongation of life span of some of the treated tumor rats is very striking.

Effect of the extract from *Glaucidium palmatum* : The extract seemed to be less

effective on tumor cells than that from *D. grayi*. Considerably low doses, such as 1 or 3 mg per 100 g did not induce any changes in tumors, while higher doses led the host animals to death by toxicity of the extract. No prolongation of life span was observed following the injection of the extract at appropriate dose levels: the treated animals lived as long as the controls did.

Discussion

Considerable work has been done with alteration of the colchicine molecule, on the one hand by investigations seeking to discover reasons for the activity of colchicine itself, and on the the other hand by inducing polyploidy in plants or in hindering tumor growth without harming the host. Among alkaloids with effects resembling those of colchicine, substances contained in podophyllin which is an extract of the powdered roots of American mandrake, *Podophyllum peltatum*, have long been subjected to etiological studies. According to the review by Kelly and Hartwell (1954), podophyllin and some active substances were also separated from Indian mandrake, *P. emodi*. In the present study, damage of the tumor cells induced by the crude extract from *Diphylleia grayi* follows the pattern described for the effects of colchicine, podophyllin and their derivatives (Sullivan and Wachsler 1947, Ormsbee 1949, Greenspan *et al.* 1950, Leiter *et al.* 1950, Cornman and Cornman 1951, Makino and Cornman 1953, Makino and Tanaka 1953, Tanaka *et al.* 1955). The cells were blocked at metaphase and then underwent pycnotic degeneration. *D. grayi* and *Glaucidium palmatum* used here belong to the same family as *P. peltatum* and *P. emodi*, from which podophyllin was isolated. The species of the Podophyllaceae are few in number and are distributed within extremely limited areas, i.e., E. Asia and E. N. America. In Japan, only two species belonging to the family have been reported to occur: the one is *D. grayi* and the other is *G. palmatum*. The present author has had the opportunity to test cytologically a possible effect of the extracts from these two species upon tumor cells. *D. grayi* acted as a marked anti-mitotic agent which resembles extracts from American and Indian species of this family, while *G. palmatum* did not produce any anti-mitotic effects on tumors.

Based on the data obtained from the present study, special attention should be directed to antitumor substance found in *D. grayi* in the future. The saline extract will be purified by various methods in order to obtain more effective substance. Determination of the chemical structure and synthesis of the substance remain for future studies.

Summary

The action of saline extracts from two Japanese Podophyllaceous plants, *Diphylleia grayi* and *Glaucidium palmatum*, was cytologically investigated in rats bearing the MTK-sarcoma III and Yoshida sarcoma and in mice bearing Ehrlich

carcinoma. The extract from *D. grayi* was found to have marked anti-mitotic effects similar to those induced by colchicine and podophyllin: blockade of mitosis at metaphase and shortening and scattering of metaphase chromosomes. The majority of cells died without proceeding beyond metaphase and this led to inhibition and regression of tumors. Marked prolongation of life span of tumor-bearing animals was observed in some of the animals treated. The extract from *G. palmatum* did not exert any anti-mitotic effect on tumors. Tumor-bearing animals treated with the extract from *G. palmatum* lived as long as control animals did.

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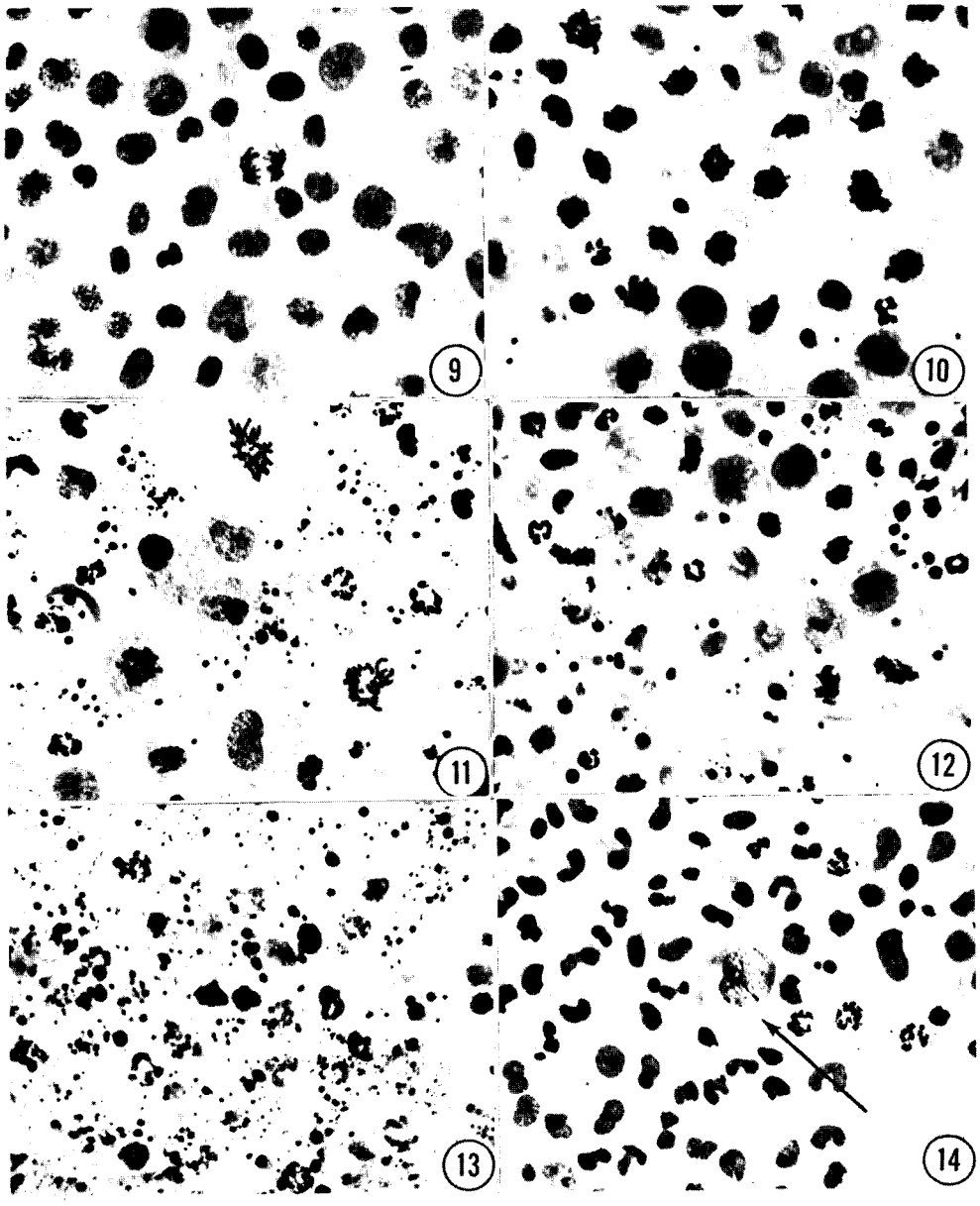

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Explanation of Plate XI

Figs. 9–14. Photomicrographs of ascites tumor cells damaged by the *D. grayi* extract. From squash preparation stained with acetic dahlia. (× 400)

Fig. 9. Untreated MTK-sarcoma III cells.

Fig. 10. Metaphase blocks of MTK-sarcoma III cells, 9 hours after treatment.

Figs. 11–12. Disintegration of tumor cells observed 12 and 15 hours after treatment.

Fig. 13. Advanced condition of cell degeneration, 18 hours after treatment.

Fig. 14. Most of the damaged cells were absorbed and inflammatory cells were markedly increased. Arrow indicates degenerating MTK-sarcoma III cell, 24 hours after treatment.