STUDY ON *GARDENIA FLORIDA* L. (*FRUCTUS GARDENIAE*) AS A REMEDY FOR ICTERUS

REPORT III

ON THE EFFECT OF THE EXTRACT OF *FRUCTUS GARDENIAE FLORIDA* ON THE AMOUNT OF BILIRUBIN IN THE PERIPHERAL LYMPH OF COMMON BILE-DUCT LIGATED RABBITS

TOJI MIWA

Department of Pharmacology, Faculty of Medicine, Gifu Prefectural University, Gifu

Received for publication December 2, 1952

INTRODUCTION

In the 1st and 2nd reports of this series, the author proved that the administration of the aqueous and alcoholic extracts of *Fructus Gardeniae floride* L. resulted in the evident increase of the secretion of bile in rabbits. It was also proved that the amount of bilirubin in the blood of bile-duct ligated rabbits, as against the control rabbit without the administration of *Gardenia florida* extract, decreased remarkably 24 and 48 hours after the administration of the extract of *Fructus Gardeniae florida*, although no remark-effect was found 6 hours after the administration.

The fact in icterus patients, the skin and conjunctiva become tinted yellow seems to indicate that the biliary dye in the blood transits into the tissue fluid. This was experimentally proved by Asakuma (1) who proved the appearance of bilirubin in the peripheral lymph (popliteal lymph) in rabbits whose common bile-ducts had been ligated. The author, therefore, examined the effect of the extract of *Fructus Gardeniae florida* on the amount of bilirubin in the peripheral lymph (popliteal lymph) of rabbits whose common bile-ducts had been ligated.

MATERIALS AND METHOD

Healthy rabbits of about 2 kg body weight were used as the experimental animals.

The lymph fluid from the popliteal space was collected by the methods iterated by Funaoka and Tsuji (2) and by Yoshida (3). The popliteal lymph gland in rabbits is commonly situated in the popliteal space, on the frontal outer edge of semimembranosus muscle, being in the rear of the inner head of gastrocnemius muscle. In the present case, a rabbit was fixed on its abdominal

position, the portion corresponding to the popliteal lymphatic gland was shaved in as large a space as possible, and the skin incised lengthwise for about 5 cm. The femoral biceps muscle that was beared was also incised for about 5 cm along its fiber, and the popliteal lymphatic gland, surrounded by adipose tissues, was easily reached by forcing back the muscle along the incision.

The artery that goes into the popliteal lymphatic gland is formed from popliteal artery in the popliteal space, and is a branch of small saphenous artery that flows onto the surface of the head of gastrocnemius muscle. This branch is accompanied by the efferent of popliteal lymphatic gland, and in the majority of cases, veins. The source of this artery that reaches the popliteal lymphatic gland forming the branch was sought out, and this gland artery and the efferent of the lymphatic gland were ligated at the same time. The movement of the hind leg a few minutes later revealed whitening and tightening of the efferent of popliteal lymph.

A glass capillary was inserted into this efferent of popliteal lymph and tied with a thread by which the lymph fluid exuded in droplets and were collected with a capillary pipette. The collection of popliteal lymph fluid was limited to once per rabbit since two collections were difficult and forced collection would have resulted in occlusion of blood and other impurities.

Lymph fluid was collected 6, 24, and 48 hours after ligation of the common bile-duct. The amount of bilirubin was determined by the Araki method (4) which is a modification of the methods of Jendrasik and Cleghorn (5) and of Jendrasik and Gróf (6), using the Pulfrich's photometer with S. 53 filter. The samples were diluted with 10% solution of caffeine and sodium benzoate and determined directly after dilution. The drug extract used was the same alcoholic extract as described in the 1st and 2nd papers of this series. The solution was prepared so as to contain 1 g of the drug per kg of rabbit weight in 5 cc of physiological salt solution, filtered, and the filtrate injected into the aural vein of a rabbit one hour after ligation of the common bile-duct.

RESULTS

1) Amount of bilirubin in the effluent of popliteal lymph gland of common bile-duct ligated rabbits

The amount of bilirubin in the effluent of popliteal lymph gland from rabbits whose common bile-ducts had been ligated was determined 6, 24, and 48 hours after ligation. As shown in Table 1, the mean value after 6 hours was 0.30 mg%, 2.21 mg% after 24 hours, and 2.97 mg% after 48 hours.

STUDY ON GARDENIA FLORIDA L.

3

TABLE 1. Amount of bilirubin in the effluent of popliteal lymph gland of common bile-duct ligated rabbits

Time after ligation (hrs.)	Rabbit No.	Sex	Rabbit weight (g)	Amount of bilirubin	
				Individual (mg%)	Mean (mg%)
6	43	♂	2 320	0.31	0.30
	44	♂	1 800	0.30	
	45	♂	1 800	0.30	
24	46	♂	2 300	2.29	2.21
	47	♂	2 200	2.31	
	48	♂	2 100	2.03	
48	49	♂	2 000	3.01	2.97
	50	♂	2 040	2.86	
	51	♀	2 100	3.06	

2) Amount of bilirubin in the effluent of popliteal lymph gland of common bile-duct ligated rabbits after injection of 5cc of physiological salt solution into the aural vein

The amount of bilirubin in the effluent of popliteal lymph gland was determined after injection of a definite amount of the alcoholic extract dissolved in 5 cc of physiological salt solution into the aural vein of rabbits whose common bile-ducts had been ligated. As a control, therefore, the amount of bilirubin in the effluent of popliteal lymph gland was determined in rabbits after injection of 5 cc of physiological salt solution alone into the aural vein one hour after ligation of the common bile-duct. The results are given in Table 2.

As can be seen from the table, the amount of bilirubin in this case does not differ significantly from that in rabbits whose common bile-ducts had been ligated but not given injection of physiological salt solution.

TABLE 2. Amount of bilirubin in the effluent of popliteal lymph gland of common bile-duct ligated rabbits after injection of 5cc of physiological salt solution into the aural vein

Time after ligation (hrs.)	Rabbit No.	Sex	Rabbit weight (g)	Amount of bilirubin	
				Individual (mg%)	Mean (mg%)
6	52	♂	2 700	0.33	0.31
	53	♂	2 800	0.37	
	54	♂	2 000	0.30	
24	55	♂	1 900	2.29	2.10
	56	♂	2 100	1.99	
	57	♂	2 000	2.04	

48	58	♂	2 300	2.98	2.96
	59	♀	2 000	2.79	
	60	♂	2 250	3.11	

3) Amount of bilirubin in the effluent of popliteal lymph gland in common bile-duct ligated rabbits after the injection of the alcoholic extract of *Fructus Gardeniae florida* into the aural vein

The alcoholic extract of *Fructus Gardeniae florida* L. was dissolved in 5 cc of physiological salt solution so as to contain 1 g of the drug per kg of body weight in rabbits whose common bile-ducts had been ligated and injected into the aural vein one hour after ligation. The amount of bilirubin in the effluent of popliteal lymph gland was determined 6, 24, and 48 hours after ligation. The results are given in Table 3.

As can be seen from the table, the mean value after 6 hours was 0.37 mg%, 1.54 mg% after 24 hours, and 2.13 mg% after 48 hours, showing that the alcoholic extract of *Fructus Gardeniae florida* clearly suppressed the appearance of bilirubin into the peripheral lymph (popliteal lymph) of rabbits whose common bile-ducts had been ligated.

TABLE 3. Amount of bilirubin in the effluent of popliteal lymph gland of common bile-duct ligated rabbits after injection of the alcoholic extract of *Fructus Gardeniae florida* into the aural vein

Time after ligation (hrs.)	Rabbit No.	Sex	Rabbit weight (g)	Amount of bilirubin	
				Individual (mg%)	Mean (mg%)
6	61	♀	2 500	0.40	0.37
	63	♂	2 140	0.46	
	63	♂	2 230	0.26	
24	64	♂	2 140	1.54	1.54
	65	♂	3 000	1.53	
	66	♀	3 060	1.56	
48	67	♂	2 480	2.03	2.13
	68	♂	2 140	2.30	
	69	♂	2 600	2.05	

SUMMARY

1. The amount of bilirubin in the peripheral lymph (popliteal lymph) fluid of rabbits whose common bile-ducts had been ligated averaged 0.30 mg% 6 hours after the ligation, 2.21 mg% after 24 hours, and 2.97 mg% after 48 hours.

2. The amount of bilirubin in the peripheral lymph (popliteal lymph) fluid of rabbits whose common bile-ducts had been ligated was practically unaffected by the injection of 5 cc of physiological salt solution into the aural vein.

3. The amount of bilirubin in similar rabbits, after the injection of the alcoholic extract of *Fructus Gardeniae florida*, dissolved in 5 cc of physiological salt solution, averaged 0.37 mg% after 6 hours, 1.54 mg% after 24 hours, and 2.13 mg% after 48 hours, showing clearly that the drug suppressed the appearance of bilirubin in the peripheral lymph (popliteal lymph) fluid in rabbits whose common bile-ducts had been ligated.

These experiments were carried out under the guidance of Prof. N. Sugihara and Assist. Prof. I. Yamagami at the Pharmacological Department of the Medical Faculty of Gifu Prefectural University. I give my sincere and heartfelt thanks to my respected teachers.

REFERENCES

- 1) ASAKUMA, S.: *Arbeiten aus der dritten Abteilung des Anatomischen Institutes der Kaiserlichen Universität Kyoto* Heft 6, 89 (1937)
- 2) FUNAOKA, S. UND TSUJI, K.: *Ibid.* Heft 4, 135 (1934)
- 3) YOSHIDA, Y.: *Ibid.* Heft 5, 83 (1935)
- 4) ARAKI, Y.: *Sogo Igaku* 4, 706 (1947)
- 5) JENDRASIK, L. UND CLEGHORN, P. A.: *Biochem. Ztschr.* 289, 1 (1937)
- 9) JENDRASIK, L. UND GRÖF, P.: *Ibid.* 297, 81 (1938)