Febuxostat, a non-purine xanthine oxidase (XO) inhibitor, is an effective drug superior to allopurinol for treatment of hyperuricemia, a risk factor of hypertension and cardiovascular diseases. In addition to its XO inhibitory effect, we aim to test its action on renal urate transporters URAT1, URATv1 and OAT10. In vitro XO activity assay was tested among 3 drugs: febuxostat, allopurinol and benzbromaron using commercial fluorometric determination kit. Febuxostat had strong inhibitory effect on in vitro XO activity with an IC50 value of 0.22 ± 0.11 nM compared to 9.20 ± 1.14 µM of allopurinol. By using Xenopus oocytes expressing urate transporters, we found that the [14C] urate transport via an apical urate exchanger URAT1, an apical organic anion transporter OAT10 and a basolateral voltage-driven urate transporter URATv1 were inhibited by febuxostat with IC50 values of 16.80 ± 1.11 µM, 46.35 ± 9.41 µM and 21.01 ± 1.33 µM, respectively. Febuxostat also showed strong interaction with organic anion transporting polypeptides (OATPs) which expressed at sinusoidal membrane of hepatocytes. Therefore, the efficacy of febuxostat in reducing serum urate level might be due to its inhibition both on xanthine oxidase (through entering the hepatocytes via OATPs) which subsequently reduced uric acid production and on renal urate reabsorption mainly via URAT1 and URATv1 transporters.